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SUSCEPTIBILITY TO CRONARTIUM RIBICOLA OF THE FOUR PRINCIPAL RIBES SPECIES FOUND WITHIN THE COMMERCIAL RANGE OF PINUS MONTICOLA[1]

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INTRODUCTION

White-pine blister rust (*Cronartium ribicola* Fisch.) was discovered in western North America in the fall of 1921 (*1*).[3] By that time it had become distributed in eastern North America over a large section of the range of northern white pine (*Pinus strobus* L.), where measures were being energetically applied for its control (*13, pp. 80–90*). Earlier reconnaissance for the disease in the western portion of the continent had given negative results, and as for some years rigid quarantines had been in effect to prevent its introduction into western territory, hope was felt that the West, with its magnificent stands of western white pine (*P. monticola* Dougl.) and sugar pine (*P. lambertiana* Dougl.) might be kept free from this destructive parasite (*13, p. 82*). Scouting in 1922, however, showed the disease to be widely distributed on ribes[4] in the coastal region west of the Cascade Range, from southwestern Washington to the northern end of Vancouver Island in British Columbia, and over a large area about 100 miles north of the international boundary in the interior of British Columbia in the region that included the towns of Canoe, Revelstoke, and Beaton. Within this range it was found on western white pine at numerous points from the Puget Sound section of Washington to the northern limits of this species about 150 miles north of the international boundary, and also at several points in the infected area in the interior of British Columbia (*10*).

Studies showed that the disease had been present on pines in some of the infected areas for several years, evidently having been introduced near Vancouver, British Columbia, about 1910, and that it was spreading and intensifying with great rapidity (*5, 10*). Evidence secured in 1922 and 1923 indicated that wind-borne aeciospores were annually infecting ribes at distances of over a hundred miles from the centers of pine infection and aeciospore production (*5, 10*). At this rate of spread it was obvious that the rust would soon invade the commercial range of western white pine, which was only a little over

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[3] Reference is made by number (italic) to Literature Cited, p. 345.

[4] The genus name *Ribes* and the common noun ribes are used in this paper to include both currants and gooseberries.

100 miles south of the infected area in the interior pine belt of British Columbia. This situation made urgent the development of measures for the protection of the pine area of northern Idaho and adjacent Montana and Washington, and control operations were promptly instituted. Spread since 1922 has borne out these assumptions, for the rust was first found there in 1928 and is now well established within the area (*11*, *20*, *21*).

One of the first problems in connection with research on the disease in the West was to determine the susceptibility of western host plants, both pines and ribes (*5*, *pp. 875–877, 881–882*). In both cases information was meager, consisting, in the case of the pines, principally of evidence of high susceptibility in *Pinus flexilis* (*13*, *p. 75*), and, for the various species of ribes, being based only on limited inoculation tests (usually in the greenhouse) in the eastern part of the United States (*13*, *pp. 18–20*).

Preliminary observations in western pine infection centers were alone sufficient to demonstrate that western white pine was highly susceptible, evidently more so than northern white pine,[5] and studies were started in 1922 to determine the character of infection on such ribes species as were present in the infected area. The primary object of the studies was to obtain information applicable to the development of measures for the control of the rust. In view of the impending spread of the disease into the commercial range of western white pine, the most urgent problem in the investigations was to obtain a knowledge of the susceptibility and telium-producing capacity of the rust on the important ribes species in that region. The present paper reports the results of these investigations.

RANGE LIMITS OF WESTERN WHITE PINES IN RELATION TO SPREAD OF THE RUST

White pine species of the region, including the infected areas and the Idaho white pine and California sugar pine stands, are western white pine, whitebark pine (*Pinus albicaulis* Engelm.), limber pine (*P. flexilis* James), bristlecone pine (*P. aristata* Engelm.), foxtail pine (*P. balfouriana* Murray), and sugar pine. These species are all known to be susceptible to the rust (*9*, *13*, *14*). Their composite botanical ranges in this region and the range of the fungus as determined in 1922 and in 1932 are shown in figure 1.

While some of these pine species are valuable only for aesthetic or watershed-protection purposes, the commercial value alone of western white pine and sugar pine must be calculated in hundreds of millions of dollars, and their ultimate value to the region in which they occur is much larger.

Western white pine occurs in two belts, which may be designated "coastal" and "interior." The coastal belt is one of scattered occurrence of the species. It extends from the southern portion of the Sierra Nevada in California, northward through western Oregon and Washington, mainly along the Cascade Range, to Puget Sound, and thence through the coastal region of northwestern Washington and southwestern British Columbia to about the northern end of Vancouver Island. The interior belt centers in the area of northern Idaho, northeastern Washington, and western Montana and stretches

[5] BOYCE, J. S. PATHOLOGICAL INVESTIGATIVE WORK. Third Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 56–60. [Mimeographed.]

northward into British Columbia for about 200 miles. The main commercial range of western white pine is confined to the States mentioned, where the lumber industry is largely dependent upon this tree for its existence.

Wild ribes are widespread over the entire territory, both inside and outside the range of the pines.

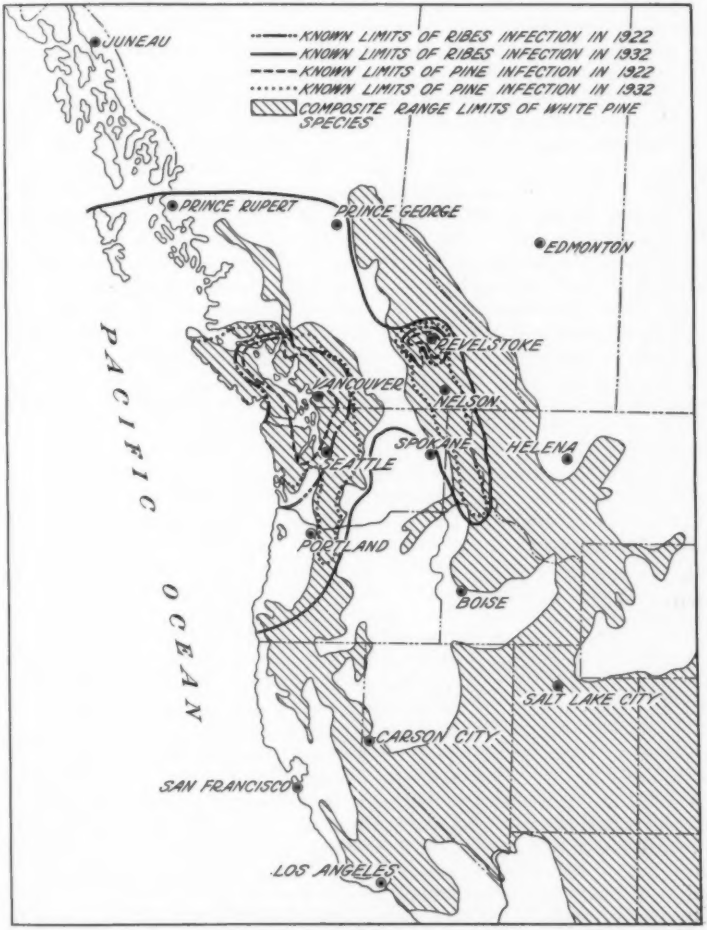

FIGURE 1.—Composite botanical range limits of white pine species in the West and known range of white-pine blister rust in 1922 and 1932.

From the distribution outlined above and from the map (fig. 1), it is evident that continuous belts of susceptible white pine exist through which the rust may be expected to spread from its known areas of distribution in 1922, over both the coastal and the interior

range of western white pine. The known range of the rust in 1932 indicates that the expected spread is in rapid progress.

RIBES SPECIES INVOLVED AND PREVIOUS STUDIES OF THEIR SUSCEPTIBILITY

At the time the rust was discovered in the West in 1921, little was known concerning the character or extent of association of ribes with commercial western white pine. By the end of 1923, however, reconnaissance [6] had indicated that for control purposes the commercial pine area centering in Idaho falls naturally into two main types—the stream type, which consists of moist stream-bank and bottom land characterized by profuse occurrence of ribes; and the timber type, which extends up and away from the stream type and in which ribes are more scattered. The most common species found in the stream type are *Ribes lacustre* (Pers.) Poir., *R. petiolare* Dougl., and *R. inerme* Rydb., the last two usually occurring in dense concentrations. The common species of the timber type are *R. viscosissimum* Pursh and *R. lacustre*. The last-named is generally more abundant numerically in both types than any of the other species. Species other than those mentioned are seldom encountered in either type.

When the study was started little was known of the susceptibility of three of the four ribes species mentioned, and nothing at all of the fourth, *R. petiolare*. Spaulding in 1922 (*13, pp. 18–20*) reported that a medium degree of infection developed on a relatively few bushes of *R. lacustre*, *R. viscosissimum*, and *R. inerme* when they were subjected to tests in the greenhouse and that slight infection developed on *R. lacustre* out of doors. Preliminary results of the present studies were reported in 1926 (*5*), showing susceptibility and telium-producing capacity to be high for *R. petiolare* and *R. inerme* and relatively low for *R. viscosissimum* and *R. lacustre*. In 1928, Hahn (*2, p. 680*) published results of greenhouse tests with these species in the East, which generally corroborated Spaulding's earlier results there and also showed *R. petiolare* to be susceptible. In neither Spaulding's nor Hahn's results, however, were any data given on the telium-producing capacity of the ribes, which is a primary consideration in control. Moreover, with the exception of *R. lacustre*, these results include nothing with regard to the reaction of the species out of doors.

Spaulding states, with respect to greenhouse inoculations (*13, p. 16*), "It was felt that greenhouse tests alone were not dependable for susceptibility data." The writers concur in this opinion. Hart (*4, pp. 930, 944–945*), working with stem rust of wheat, mentions instances in which tests conducted under the unnatural conditions of the greenhouse have given results contradictory to those secured in the field. Because of the wide variety of conditions found in nature, it was believed that only comprehensive studies out of doors under different site conditions could give a reliable index to the susceptibility of such variable hosts as ribes. These hosts have different growth forms within each species which differ as widely from one another in susceptibility as does one species from another. To reproduce these different forms and conditions on a sufficient scale in the greenhouse

[6] By the western branch of the former Office of Blister Rust Control, Bureau of Plant Industry, now comprised in the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

would be quite out of the question. Accordingly, the studies reported in this paper were conducted out of doors.

METHODS

DEVELOPMENT OF STUDY METHODS

Determination of the general susceptibility under natural conditions of the four most common species of ribes in the Idaho-Montana-Washington area, has been one of the principal objectives of studies of the rust in the West. In the reaction of any species of ribes to *Cronartium ribicola*, two features are of outstanding importance in control: (1) The relative importance of a species and its various forms in the long-distance spread and local establishment of the rust in disease-free regions; (2) its ultimate capacity, after the rust has become established in a locality, for spreading infection to associated pines. The first logical step was to obtain a measure of the general susceptibility of the species, both to infection by aeciospores and intensification by urediospores, together with its capacity for producing telia from which the pine-infecting sporidia are developed.

After the inauguration of these studies, information became available (*15, p. 915*) which indicated that the relationship between teliospore production and the production of sporidia was not the same on all ribes species, since under natural conditions teliospores remained viable for much longer periods on some species than on others. Also, the possible existence of varying degrees of longevity and virulence in sporidia from different ribes hosts may be inferred from the results of studies reported at about the same time (*12, p. 589; 16, p. 419; 22, pp. 508–509*). The potential pine-damaging powers of various ribes, therefore, cannot be considered a direct function of their capacity for teliospore production, but until further experiments provide more definite information on this point an approximately constant relationship must be assumed.

Before any satisfactory tests of ribes susceptibility could be made, however, it was necessary first to devise a simple method of recording infection data that would permit the averaging of results and a ready comparison of the data of different years and places. The system used by Spaulding (*13, p. 17*) of differentiating the infection merely by the terms "light", "medium", and "heavy" was inadequate, because it did not permit the averaging of results and because the definitions of such terms vary considerably with the individual observer. Furthermore, the present study was concerned primarily with the telium-producing capacity of the ribes as a preliminary measure of their potentialities for transmitting the disease to the pines. Spaulding's system gave no measure of this capacity. Systems devised by investigators of other rusts (*7, 17, 19*) were equally inapplicable because of differences in the character of hosts and study objectives.

Concentrating on the features primarily essential to control, as given at the beginning of this section, and working with naturally infected plants in the vicinity of infected pines in 1923 and 1924, the following system of study was devised:

(A) By individual bushes:
  (1) Count of leaves produced.
  (2) Count of leaves infected.
  (3) Estimate of percentage of surface infected on infected leaves.
  (4) Count of rust-killed leaves dropped from bushes.

(5) Estimate of percentage of infected surface which—
  (*a*) Bore uredia.
  (*b*) Had borne uredia but had died before producing telia.
  (*c*) Had borne uredia but had fallen before producing telia.
  (*d*) Was necrotic, i. e., dying before producing any spore stages.
  (*e*) Bore telia.

(B) By groups of bushes:
  (1) Counts and estimates of leaves by individual bushes.
  (2) Count of bushes infected.
  (3) Estimate of percentage of leaves infected on infected bushes as a group.
  (4) Same as under 3, system A, for the group as a whole.
  (5) Estimate of percentage of rust-killed leaves dropped for the group as a whole.
  (6) Same as under 5, system A, for the group as a whole.

In addition to the observations outlined above, descriptive notes were taken on any abnormalities encountered and on the general character of infection and sporulation. Representative specimens of the infection were collected for each species and form to serve as permanent records supplementary to the data taken at each examination.

These systems were found to permit rapid collection of data and to give readily comparable results of sufficient accuracy for practical purposes. In general, after a little experience, percentage estimates of the different recorders were numerically within 1 to 5 or less of one another in the lower and upper percentage levels, and within less than 10 of one another at percentages around 50. Errors were usually compensating. System A was used where comparison between individual bushes was necessary. Where comparison between groups was the primary consideration, system B was used with such modifications or additions as were desired for individual comparisons. This latter system was far more rapid than the former in covering large numbers of bushes, and, although somewhat less accurate as far as any one group of plants was concerned, it decreased the probable error in the final results by permitting the use of a much larger basis.

Collection of the data by the systems just described made it possible to compute these data by simple arithmetical methods. In this connection the two things of primary consideration in the study were (1) percentage of total leaf surface infected, which was obtained by multiplying the percentage of the total number of leaves infected on a group of plants by the average percentage of surface infected on the infected leaves, and (2) the percentage of total leaf surface bearing telia, obtained by multiplying the percentage of total leaf surface infected by the percentage of infected surface bearing telia.

SELECTION OF STUDY AREAS

Prior to the inauguration of this study the wide spread of the rust by wind-borne aeciospores was practically confined to the extremely susceptible cultivated black currant (*Ribes nigrum* L.). The disease was very scarce and seldom found on other ribes species outside the general vicinity of infected pines. Of the four ribes species in question, *R. lacustre* was at that time the only one found close to infected pines in sufficient numbers to give any definite information of its susceptibility. Plants of the four species were therefore introduced and planted near infected pines in the spring of 1923. These plantings were made mainly with cuttings and gave but poor survival and results.

During the season of 1923 the rust was widely distributed by the wind to cultivated black currant plantings over the dry belt of British Columbia, lying between the coastal and interior white pine belts. This belt contains an abundance of all four of the ribes species studied, which are well adapted to this region and grow prolifically. Since pines were absent from the area, since spread of the rust from ribes to ribes is slight as compared with the distance of spread from pines to ribes, and since it was evident that the rust did not overwinter on ribes in the region, this belt offered a splendid opportunity to make inoculation tests of naturally growing plants of these species under comparable conditions without any danger of permanently establishing the disease or hastening its spread. Tests were accordingly begun there in 1924 near Kelowna, British Columbia, where the rust had been abundant on cultivated black currants in 1923. To this study locality were added two others in the same belt in 1926, one each near Summerland and Oliver at elevations similar to Kelowna (table 1), and

TABLE 1.—*Description of study areas*

Area	Elevation	Soil[1]	Slope and exposure	Vegetation[2]	Remarks
Interior British Columbia:					
Near Kelowna:	*Feet*				
Swamp area	1,150	P, t	Flat	G, S, A, B	Wet, poorly drained flat. Somewhat more rain than at Kelowna.
Canyon Creek area.	1,900	L, G, h	Gentle, W	B, A, T, F, C	Fairly moist but well drained.
Summerland	1,200	V	Gentle, SE.	B, M, S	Moist. Some springs. Well drained.
Oliver	1,000	V, S, P	Flat	S, A	Swampy in some places.
Near Osoyoos:					
Haynes Creek area.	2,500	V, S, L	do	A, C, B, Y, P, F, L.	Summer showers more frequent than at Oliver (nearest weather station).
Nine Mile Creek area.	3,200	V, S, H, p	do	Y, F, E, A, M, L.	Semiswampy in places. More rainfall than at Haynes Creek.
Camp McKinney	4,500	S, H, g	do	P, L, S, A	Much more rain than at Rock Creek (nearest weather station).
Coastal British Columbia:					
Garibaldi (Daisy Lake):[3]					
Chance Creek area.	1,100	S, H, g	do	W, H, F, A, C	
Lake area	1,100	S, H, L	do	W, F, A, C, H	
Near Mile 72, Pacific Great Eastern Ry.:					
Lower Trail area.	2,200	R, H, L	Steep, S	F, A, M, B	Moist in patches but well drained. Nearest weather station, Owl Creek.
Tenquil Valley area.	4,800	V, L	Steep, SE.	H, W, E, I, K, A.	Moist, swampy for *Ribes lacustre*. Well drained for *R. viscosissimum*. Nearest weather station is at Owl Creek.

[1] Key: G, gravel; H, humus; L, loam; P, peat; R, rocky; S, sand; T, silt; V, volcanic. Capital letters indicate abundance.

[2] Key: A, alder; B, birch; C, cottonwood; E, Englemann spruce; F, Douglas fir; G, swamp grasses; H, western hemlock; I, alpine fir; K, whitebark pine; L, larch; M, maples; P, lodgepole pine; S, willow; T, western red cedar; W, white pine; Y, yellow pine.

[3] Formerly Daisy Lake; name recently changed to Garibaldi.

two at higher elevations at the edge of the dry belt on Anarchist Mountain, east of Osoyoos, British Columbia (Haynes Creek and Nine Mile Creek). An additional area was established in 1927 near

Camp McKinney, several miles northeast of Nine Mile Creek. The last three areas, particularly the one at Nine Mile Creek, approach more closely the conditions of the interior white pine stands than do those at Kelowna, Summerland, and Oliver. Most of the white pine region plant species other than white pine are present, and the climate is fairly similar to that of much of the pine region.

During the course of these tests in the interior dry belt, a parallel series of studies involving species of ribes important in the coastal region of British Columbia was carried out at various natural infection centers in the latter region. Although *Ribes petiolare* and *R. inerme* do not occur there, *R. lacustre* and *R. viscosissimum* are native and were included at four of the study areas. At three of these areas, aeciospores from adjacent infected pines were so abundant that no inoculations were necessary because natural infection on the ribes approximated that produced by inoculation. In the fourth area, nearby aeciospore production was insufficient and the plants were inoculated. The studies of *R. lacustre* and *R. viscosissimum* in the coastal region served not only for comparison of the susceptibility of these two species occurring in the interior region, but also afforded an opportunity to observe the effect of somewhat different climatic conditions on the development of the rust on these two hosts tested in both regions.

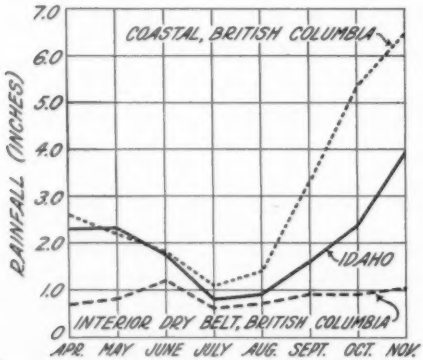

FIGURE 2.—Average rainfall during growing season. Graph prepared from United States and British Columbia weather records up to and including 1933. Records from the following stations were used. For Idaho: Avery, Potlatch, Prichard, St. Maries, Priest River Experiment Station, and Pierce. For interior British Columbia: Kelowna, Summerland, Oliver, and Rock Creek. For coastal British Columbia: Garibaldi, Owl Creek (Pemberton Hatchery), and Vancouver. The latter station has been included because rainfall there is about average for a rather large portion of the coastal region. Averages for the various stations represent periods ranging from 10 to 33 years.

A condensed description of the various areas is given in table 1. Rainfall during the growing season at weather stations nearest the study areas and at several points in the Idaho white pine region is shown graphically by months in figure 2. It will be seen that precipitation during this period was somewhat more abundant near the coast and in Idaho than in interior British Columbia. Relative humidities also averaged somewhat lower in the latter region. As will be shown later, however, these differences had little effect on the susceptibility of ribes to the rust.

INOCULATIONS AND EXAMINATIONS

The number of bushes of each species tested on areas in interior British Columbia is shown by years in table 2. These figures include the three growth forms—open, part-shade, and shade—of ribes studied. The studies involved 5,098 tests of a total of nearly 3 million leaves on 2,740 different bushes. Additional details regarding the basis for each species and form are included later under Results and Discussion.

TABLE 2.—*Bushes of Ribes species used each year in the tests on study areas in interior British Columbia* [1]

Species and area	Ribes bushes tested in—				
	1924	1925	1926	1927	1928
R. petiolare:	*Number*	*Number*	*Number*	*Number*	*Number*
Swamp	93	135	153	76	
Summerland			18	16	
Oliver			82		
Nine Mile Creek			148	144	145
Haynes Creek			96	50	
Camp McKinney				30	30
R. inerme:					
Swamp	90	104	166	66	
Oliver			66		
Nine Mile Creek			164	159	159
R. viscosissimum:					
Canyon Creek	99	98	184	280	150
Camp McKinney				100	100
R. lacustre:					
Swamp	52	24	48	47	
Canyon Creek	93	99	216	315	157
Nine Mile Creek			165	164	164
Haynes Creek			105	48	
Camp McKinney				100	100

[1] In the majority of cases the number of bushes tested in a given year includes some of those tested in a previous year or years.

All bushes tested in the interior dry belt and many of those on areas near the coast were artificially inoculated. Under overcast skies and when a good rain appeared imminent, the inoculum was applied to the under surface of the leaves with the aid of a paper bag. The bag was partly filled with freshly sporulating cankers and then used as a bellows for puffing the spores into the bushes. This method assured a fairly even distribution of spores over the leaves. Each bush was given a heavy shower of aeciospores, comparable, it was believed, to that which might have occurred naturally had the bushes been growing in close proximity to heavily infected pines. Inoculations were made in the spring, generally when ribes leaves were young and highly susceptible (*6*), and were repeated whenever weather immediately following the first inoculation was not favorable to infection. Leaf development and weather conditions accompanying each inoculation of plants in the interior are summarized in table 3.

The test plants were examined at fairly regular intervals during each growing season, data being taken as previously outlined under Development of Study Methods. During 1924 and 1925, system A (data by individual bushes) was used. Tests of greater numbers of plants during subsequent years necessitated the use of system B (estimates by groups) except in a few special instances where differences between individual bushes of the same species were being studied. During 1924, when data were recorded approximately once each month, great changes in extent and character of infection occurred between examinations. Since at that time relatively little was known about the development of the rust on these species of ribes, the interval between examinations was shortened to 1 or 2 weeks in 1925. In 1926 and 1927, data were taken every 3 or 4 weeks; and in 1928, every month or 6 weeks. Intensification of the rust by urediospores is irregular, new infection almost always developing only after rainy periods, but knowledge of the incubation period (*13, p. 40*), together

with a fairly complete record of weather conditions, generally made it possible to synchronize examinations with the culmination of each wave of intensification.

TABLE 3.—*Artificial inoculations of Ribes species on study areas in interior British Columbia, 1924–28*

Year	Areas inoculated	Date	Leaf development [1]				Weather following inoculations
			R. petiolare	*R. inerme*	*R. viscosissimum*	*R. lacustre*	
1924	Swamp and Canyon Creeks.	May 5–6	3	3	3	3	Warm and dry.
	do	May 16–17	4	4	4	4	Hot and dry.
	do	May 31					Showers in evening.
1925	do	May 18	3–4	4	4	4	Heavy dew but no rain until May 28.
	do	May 29					Good showers.
1926	do	May 4	4	4	4	4	Showers each night.
	Summerland and Oliver	May 5	4	4			Showers nights, May 5, 6.
	Nine Mile and Haynes Creeks.	May 6–7	[2] 3 (1–2)	3 (2)		3	Unsettled; rainy nights.
	All 1926 areas	May 10–12					Clear to cloudy; no rain until evening of May 13.
1927	Swamp and Canyon Creeks.	May 16	2 (3)	3 (3–4)	3–4	3–4	Good showers.
	Summerland	May 17	3				Do.
	Nine Mile and Haynes Creeks.	do	2	3–4	2	3–4	Do.
	Nine Mile Creek	June 5	3	4		4	Good showers June 7, 8.
	Camp McKinney	June 6	1–2		3–4	3	Good rain.
1928	Canyon Creek	May 11			4	4	Very little rain.
	do	May 21			5	4	Light shower.
	do	May 28			5	5	24 hours of rain.
	Nine Mile Creek	May 30	4	4		4	Rainy.
	Camp McKinney	do	1–3		3	3	Do.
	Nine Mile Creek	June 7	4	4		4	Thunder shower June 8.
	Camp McKinney	do	3		3	3	Do.

[1] Leaf-development stages are classified as follows: 1, Just breaking from bud; 2, up to ½ apparent full size; 3, up to ¾ apparent full size; 4, oldest leaves have reached apparent full size; 5, many leaves have reached apparent full size and are beginning to harden.

[2] Numbers in parentheses refer to shade-form plants, other numbers to open form. Where no distinction is made, development was approximately the same in both forms.

RESULTS AND DISCUSSION

RIBES GROWTH FORMS

Test plants of each species were divided into three classes—open form, part-shade form, and shade form—data from which were kept separate throughout the study. Bushes entirely exposed to the sun during most of the day were classed as open form, while those receiving little or no direct sunlight made up the shade-form group. Part-shade plants were approximately intermediate in exposure to sunlight.

Since infection is favored by succulence of foliage (*2, p. 666; 6; 13, pp. 45–46*), the differences in susceptibility found between the three growth forms are largely explained by the differences in the character of their leaf development. Open-grown ribes are usually short and dense. Their leaves, which are relatively small and thick, generally become quite tough within 4 to 6 weeks after breaking from the buds. Shade-form plants are more rambling and loosely branched, with relatively large thin leaves. Although these leaves toughen to a certain extent, the process is slow and never reaches a degree comparable to that of open-form leaves (*6, p. 97*). Differences in time and degree of foliage maturation induced by exposure are very striking in both *Ribes inerme* and *R. lacustre*. Leaves on open and shade *R. petiolare*

differ appreciably, but infection does not seem to be affected by leaf character to the same extent here as in the other species. New leaves usually continue to appear on all species and forms until about the first of July, after which leaf production is negligible.

SEASONAL DEVELOPMENT OF THE RUST ON RIBES

Characteristic examples of the development of the rust are given graphically in figure 3, where percentages of total leaf surface infected and total leaf surface bearing telia are shown throughout the season for shade-form plants at Nine Mile Creek in 1926 and at Camp McKinney in 1928. Examinations are indicated by small circles, and the curves between examination dates were sketched from general knowledge of the relation between weather conditions and uredial intensification. Percentages at any point in figure 3 represent accumulations, to that date, of all infection, both living and dead, and the figures throughout the remainder of this paper are based on the accumulated totals of such infection at the end of each season.

These examples are fairly typical of the rust behavior on open-form as well as on shade-form bushes. It will be seen that during the early part of the season the disparities between species offer little or no indication of their relative susceptibility as it finally appears. The great bulk of the infection on both *Ribes petiolare* and *R. inerme* generally develops from late June to mid-August, through uredial intensification. In the other two species (*R. lacustre* and *R. viscosissimum*) this midsummer pyramiding of infection is usually relatively unimportant.

GENERAL SUSCEPTIBILITY OF THE FOUR SPECIES

Total average infection and telial production on all test bushes in interior British Columbia are shown in table 4 and figure 4. *Ribes petiolare* stands far ahead of the other species in both respects. *R. inerme*, also, is a very congenial host, but *R. viscosissimum* and *R. lacustre* are relatively resistant and the latter is particularly low in the production of telia. Differences between forms are as pronounced as those between species, shade and part-shade plants showing more infection and bearing more telia than corresponding open-grown bushes. Infection is probably facilitated by more favorable moisture conditions in the shade as well as by the greater succulence of shade-form leaves.

TABLE 4.—*Basis of tests and summary of infection on inoculated Ribes plants, interior British Columbia, 1924–28*

Species	Basis									Average total leaf surface [1]					
	Leaves tested			Bushes			Tests [2]			Infected			Bearing telia		
	Open form	Part-shade form	Shade form	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
R. petiolare	603, 533	121, 372	169, 004	234	150	346	381	250	585	22.6	36.1	34.8	20.3	35.6	32.7
R. inerme	366, 710	156, 363	188, 161	218	85	262	384	185	405	13.9	22.4	23.0	4.9	18.8	18.7
R. viscosissimum	89, 226	------	66, 159	256	----	269	507	----	504	6.6	----	11.5	3.5	----	7.3
R. lacustre	617, 127	235, 568	305, 309	306	132	482	687	259	951	3.4	4.3	8.1	.4	.7	2.9

[1] Averages computed on total-leaf basis.
[2] The same bush tested in each of 2 years equals 2 tests, tested 3 years equals 3 tests, etc.
[3] Tested during 3 years only (1926, 1927, and 1928).

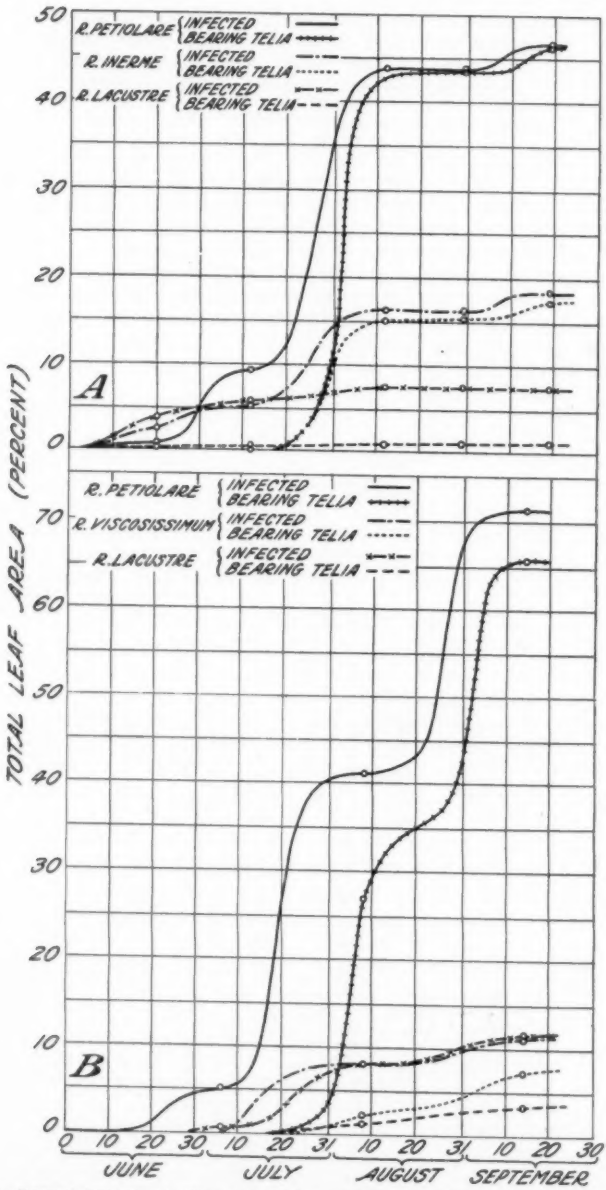
R. PETIOLARE {INFECTED / BEARING TELIA
R. INERME {INFECTED / BEARING TELIA
R. LACUSTRE {INFECTED / BEARING TELIA
A
R. PETIOLARE {INFECTED / BEARING TELIA
R. VISCOSISSIMUM {INFECTED / BEARING TELIA
R. LACUSTRE {INFECTED / BEARING TELIA
B
TOTAL LEAF AREA (PERCENT)
0 10 20 30 40 50
0 10 20 30 40 50 60 70
0 10 20 30 10 20 31 10 20 31 10 20 30
JUNE JULY AUGUST SEPTEMBER

FIGURE 3.—Seasonal development of the rust on shade-form ribes. *A*, At the Nine Mile Creek area in 1926. Basis: *R. petiolare*, 50 bushes and 20,505 leaves; *R. inerme*, 50 bushes and 37,540 leaves; *R. lacustre*, 50 bushes and 35,950 leaves. *B*, At the Camp McKinney area in 1928. Basis: *R. petiolare*, 30 bushes and 4,800 leaves; *R. viscosissimum*, 50 bushes and 8,450 leaves; *R. lacustre*, 50 bushes and 14,400 leaves.

Data from 144 susceptibility tests, involving 34,208 leaves of *Ribes viscosissimum*, and from 1,214 tests, involving 420,756 leaves of *R. lacustre*, in the coastal region of British Columbia, generally substantiate the results obtained from the tests in the interior for these species. Many of the coastal inoculations were unsuccessful because of attendant unfavorable weather, but in all cases where either natural or artificial infection by aeciospores was secured under favorable conditions the final infection was approximately similar to that recorded in table 4. It therefore appears that the greater precipitation and higher relative humidity characteristic of the coastal region have little effect upon uredial intensification, and that the behavior of the rust on ribes within the commercial range of western white pine, where spring and summer moisture conditions are roughly intermediate between

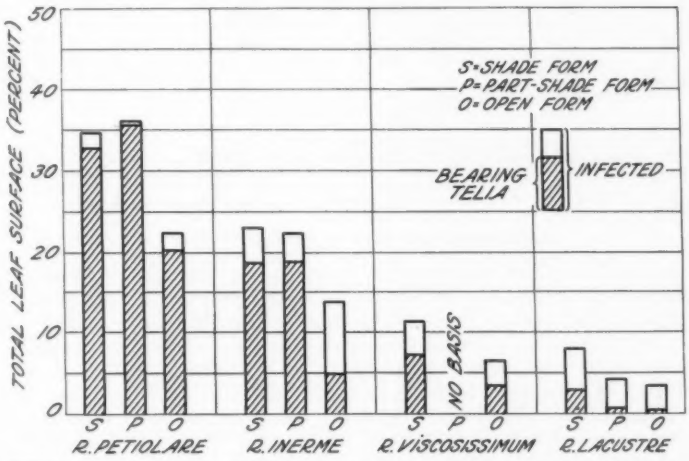

FIGURE 4.—Average infection and telial production on ribes tested in interior British Columbia.

those of the interior and coastal test areas, will not differ significantly from that herein described. This assumption is substantiated by preliminary results from the north Idaho pine region, which are presented in table 5.

The Idaho infection averages for *Ribes petiolare* are similar to the corresponding British Columbia averages, but those for *R. viscosissimum* and *R. lacustre* are somewhat lower than the ones in British Columbia. The Idaho data are for 2 years only, but on this basis, together with observations made at several other localities in the State during 5 seasons, it is believed that ribes infection will not vary greatly from that in British Columbia. The Idaho studies included 562 tests involving nearly 400,000 leaves.

TABLE 5.—*Infection on naturally infected Ribes, Idaho, 1933 and 1934* [1]

Place and area	Species	Year	Basis						Total leaf surface					
			Bushes			Leaves			Infected			Bearing telia		
			Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Fernwood, Crystal Creek.	*R. petiolare*	1933	20	25	----	24, 510	14, 215	------	34. 40	40. 20	----	33. 80	39. 50	----
		1934	20	25	----	15, 145	8, 950	------	12. 09	27. 17	----	11. 99	27. 14	----
	R. viscosissimum.	1933	25	25	25	4, 195	5, 045	7, 785	. 53	1. 01	0. 66	. 17	. 52	0. 61
		1934	25	25	25	5, 761	4, 920	11, 878	. 42	. 83	. 76	. 36	. 79	. 74
	R. lacustre	1933	25	25	25	51, 895	29, 645	33, 725	. 18	. 73	2. 42	. 02	. 26	1. 04
		1934	25	25	25	44, 095	62, 885	33, 135	. 03	. 11	. 72	. 004	. 04	. 44
Elk River, Ruby Creek.	*R. lacustre*	1933	----	25	----	------	6, 725	------	------	2. 06	----	------	. 70	----
		1934	----	25	----	------	13, 351	------	------	3. 60	----	------	. 99	----
	----do----	1933	----	25	----	------	2, 289	------	------	6. 66	----	------	4. 83	----
		1934	----	22	----	------	3, 571	------	------	. 92	----	------	. 48	----
Clarkia, St. Maries River.	*R. petiolare* [2]	1933	----	25	----	------	3, 625	------	------	36. 00	----	------	32. 90	----
	R. inerme [2]	1933	----	25	----	------	3, 250	------	------	7. 50	----	------	4. 68	----
	R. lacustre [2]	1933	----	25	----	------	5, 875	------	------	3. 30	----	------	1. 08	----

[1] All the ribes plants for which data are given were growing in close proximity to heavily infected pines.
[2] Only 1 examination was made of these bushes, on Aug. 17. Shortly after that date the bushes were destroyed by blister-rust eradication crews. No doubt considerably more rust would have developed on them, for intensification of the disease was taking place in this vicinity until about the first part of October. Data were taken on all the other groups for both years until intensification had ceased.

REACTION OF INDIVIDUAL BUSHES WITHIN GROUPS

Individual ribes bushes of a given species frequently show a very marked difference in their reaction to white-pine blister rust. The extent of this variation, as illustrated by the differences in percentage of leaf surface infected within groups of the four species and their forms under consideration, is indicated in table 6, which shows the number of bushes of each group in the different infection classes.

The individuals composing each group of these bushes occurred within small limits, the radii of which seldom exceeded 50 feet. On each area the ground was nearly level and in general the environmental conditions to which the bushes were subjected appeared fairly uniform. There was no evidence, therefore, that variations in environment were great enough to affect significantly the reactions of the individual bushes to the rust.

Practically all infection estimates during and after 1926 were made by groups rather than by individual bushes; data are therefore presented for only the 1924 and 1925 tests. However, since no bush was used more than once during the 2 years, these data are based on a good assortment of individuals. They may be considered fairly representative of the degree of variability usually encountered within test groups throughout the study, in spite of the fact that infection averages differed appreciably from the final figures for all groups as shown in table 4.

Within the groups represented in table 6, individual infection ranged from less than 1 percent of the leaf surface in all forms to about 70 and 75 percent, respectively, for the open and shade forms of *Ribes petiolare;* 75 and 90 percent, respectively, for the open and shade forms of *R. inerme;* 45 and 70 percent in *R. viscosissimum;* and about 10 and 70 percent in *R. lacustre.* These maxima are in most cases still considerably below the greatest actually recorded during

TABLE 6.—*Variation of the extent of infection within groups of inoculated Ribes, interior British Columbia, 1924 and 1925*

Leaf surface per bush infected (percent)	Bushes of species and forms tested falling in the indicated susceptibility class																					
	R. petiolare					*R. inerme*						*R. viscosissimum*				*R. lacustre*						
	1924		1925			1924		1925				1924		1925		1924			1925			
	Open	Shade	Open		Shade	Open	Shade	Open		Shade		Open	Shade	Open	Shade	Open	Shade		Open	Shade		
			No. 1	No. 2				No. 1	No. 2	No. 1	No. 2						No. 1	No. 2		No. 3	No. 4
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
0	0	11	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0
Trace–1	5	5	16	0	10	1	0	0	4	1	3	5	8	14	2	19	1	24	38	1	17
1.01–5	3	3	3	3	32	0	1	6	3	7	1	12	15	17	12	6	3	23	10	6	17
5.01–10	2	8	0	1	6	0	0	11	3	7	7	7	12	11	8	2	5	7	1	4	7
10.01–15	0	4	1	2	1	0	2	3	1	7	1	5	5	6	5	0	3	3	0	3	6
15.01–20	0	5	1	3	0	1	4	2	0	7	1	5	3	1	7	0	2	5	0	4	1
20.01–25	0	2	0	3	0	1	3	3	2	1	0	4	5	1	2	0	10	1	0	2	0
25.01–30	2	1	1	8	0	2	4	1	0	2	0	6	1	0	5	0	2	0	0	2	0
30.01–35	2	1	5	7	0	6	8	2	0	1	0	2	5	0	2	0	6	1	0	2	1
35.01–40	6	2	5	3	0	5	13	2	0	1	0	0	0	0	1	0	5	0	0	0	0
40.01–45	6	2	6	1	0	1	6	0	0	0	0	1	1	0	2	0	8	0	0	0	0
45.01–50	1	1	3	2	0	3	3	0	0	1	0	0	1	0	1	0	4	0	0	0	0
50.01–55	5	0	3	4	0	2	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
55.01–60	6	3	1	1	0	1	2	2	0	0	0	0	1	0	0	0	1	0	0	0	0
60.01–65	3	0	2	0	0	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
65.01–70	3	0	0	0	0	1	5	0	0	0	0	0	0	0	1	0	1	0	0	0	0
70.01–75	0	1	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75.01–80	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.01–85	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85.01–90	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90.01–95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95.01–100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	44	49	48	38	49	27	63	33	14	35	13	41	58	50	48	27	52	66	49	24	49
Average percentage of leaf surface infected[1]	*Pct.* 39.4	*Pct.* 20.1	*Pct.* 24.3	*Pct.* 29.7	*Pct.* 2.5	*Pct.* 41.4	*Pct.* 43.8	*Pct.* 17.3	*Pct.* 10.6	*Pct.* 13.5	*Pct.* 6.1	*Pct.* 10.4	*Pct.* 12.4	*Pct.* 5.0	*Pct.* 16.4	*Pct.* 1.1	*Pct.* 29.6	*Pct.* 4.6	*Pct.* 0.7	*Pct.* 13.1	*Pct.* 4.6

[1] Averages computed on bush basis.

the course of this study. Individual plants of *R. petiolare* have frequently been observed with over 90 percent of the leaf surface infected. In one case at Camp McKinney, in 1928, a group of 30 shade-form bushes of *R. petiolare* averaged 71.3 percent of the leaf surface infected. The shade and part-shade forms of this species have already been shown by the averages in table 4 to be the most susceptible of all the species and forms represented in the tests. The data in table 6 for the shade-form group tested in 1925 are therefore decidedly atypical.

The maxima for *Ribes inerme* in some of the groups other than those at present under consideration sometimes exceeded 90 percent of the surface infected, although the leaves of this species are much more susceptible to injury from the rust than are those of *R. petiolare*, and usually wilt and fall to the ground before such a degree of infection is reached. Occasional bushes of *R. viscosissimum* and the shade form of *R. lacustre* also ranged considerably higher in percentage of leaf surface infected than shown, while bushes of the open form of *R. lacustre* frequently exceeded the indicated maximum by large margins. For group 1 of the shade form of *R. lacustre* in table 6, however, the average degree of infection and the extent to which the bushes range into the heavier infection classes are far greater than usually encountered in this form and species.

The distribution of the samples of *Ribes petiolare* shown in table 6 indicates that this species is made up of at least two strains, one resistant and the other highly susceptible. Because of insufficient data, the values in this table do not give a correct impression of the proportion in which the resistant strain occurs. While exact figures are not available, observational experience throughout the study indicated that not more than 10 or 15 percent of the bushes possessed resistance of that order, the rest ranging mainly from moderate to very high in susceptibility.

Ribes inerme (table 6) shows a similar though less marked tendency toward division into strains. *R. viscosissimum* exhibits a dispersion of the bushes over a considerable range of susceptibility, with a concentration in the resistant classes. The open form of *R. lacustre* shows a characteristic concentration of the bushes in the extremely resistant classes. Disregarding the atypical case of group 1, which has already been mentioned, the shade form shows a typically light infection, although individual bushes may exhibit a fairly high susceptibility.

General observations indicated that the technique employed in these tests largely, if not entirely, eliminated the effect of irregularities in the inoculations. Infection resulting directly from aecial inoculations is of relatively small extent in all species (*6, p. 109*) (see also fig. 3), the great bulk of infection on the susceptible plants developing later in the season by means of uredial intensification. This is usually the case even when bushes are showered with great quantities of aeciospores. All of the groups now being considered, and practically all of the other plants tested during the study, were inoculated more heavily than necessary to secure approximately maximum initial infection. Therefore, and since pains were taken to secure as even distribution of the inoculum over the foliage as possible, it may be assumed that variability of individual bushes in extent of infection was not the result of inequalities in inoculation.

It will be seen (table 6) that for no species can infection be considered approximately uniform, even when bushes are subject to the same general environment. Because of this variability, results secured from small numbers of bushes are extremely unreliable indices of general susceptibility. When the effects of dissimilar environments and wide local and annual differences in weather are considered, it is obvious that a large basis is necessary for the determination of average susceptibility.

INHERENT DIFFERENCES WITHIN SPECIES

Evidence of inherent differences in susceptibility between bushes of the same species, particularly *Ribes petiolare*, has already been pointed out in the discussion of table 6. The extremely light infection of the shade group of this species at the Kelowna swamp in 1926 and 1927 may be considered as additional evidence of the existence of resistant strains in this species.[7] Besides this group, there also occurred at this same area the five groups of *R. petiolare* included in table 6 and three others composed of open-form bushes, or a total of nine separate groups. With the exception of one very lightly infected group, all open-form groups became abundantly infected every year they were tested, while only one of the shade-form groups developed infection enough to be termed moderate in amount. In every group, however, there were resistant and susceptible individuals. Some of these individuals were inoculated as many as 4 years and consistently maintained their resistance or susceptibility during that time. Also, at all the other areas where *R. petiolare* occurred there were individuals exhibiting these characteristics, and this was true for the other species tested at the various localities.

All nine groups of *Ribes petiolare* mentioned above occurred on a relatively small portion of a large level swamp formed by the gradual recession of a lake. The resistance shown by the shade group in 1926 and 1927, therefore, cannot be ascribed to a difference in site factors but must be considered as probably inherent. Individuals in this group, in many cases apparently derived by layering from a common origin, generally became infected either very lightly or not at all, while others, closely associated or even intermingled with the resistant bushes, were much more severely attacked. Hahn (*2, table 2*) records the occurrence of several immune plants of *R. petiolare* in a series tested in the greenhouse, and in another publication (*3*) clearly demonstrates that wide differences in susceptibility exist between horticultural varieties of both *R. nigrum* L. and *R. sativum* Syme.

In an effort to obtain additional information regarding the extent of inherent differences in susceptibility within the same species, a number of bushes previously found to range from resistant to highly susceptible were selected from each species and form and tested during a period of 2 years or more. Each group was composed of plants growing fairly close to each other and under environmental conditions as similar as possible. Results are illustrated in figures 5 and 6, where data from four representative groups are presented graphically.

[7] See section entitled "Local and annual differences in infection."

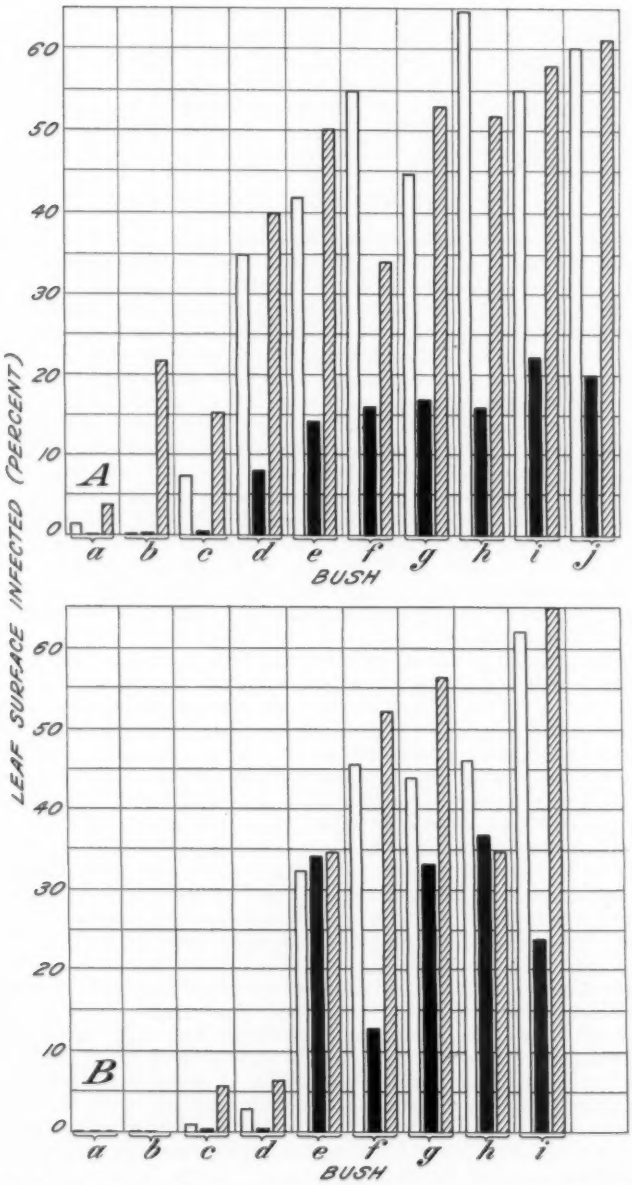

FIGURE 5.—Susceptibility of individual open-form *Ribes petiolare* bushes. 1924 (or 1925) infection, unshaded; 1927 infection, solid; 1928 infection, diagonally hatched. Two separate groups of bushes (*A*, *a–j*, and *B*, *a–i*), are represented in the figure. These groups occurred about 150 yards apart within a large swampy area.

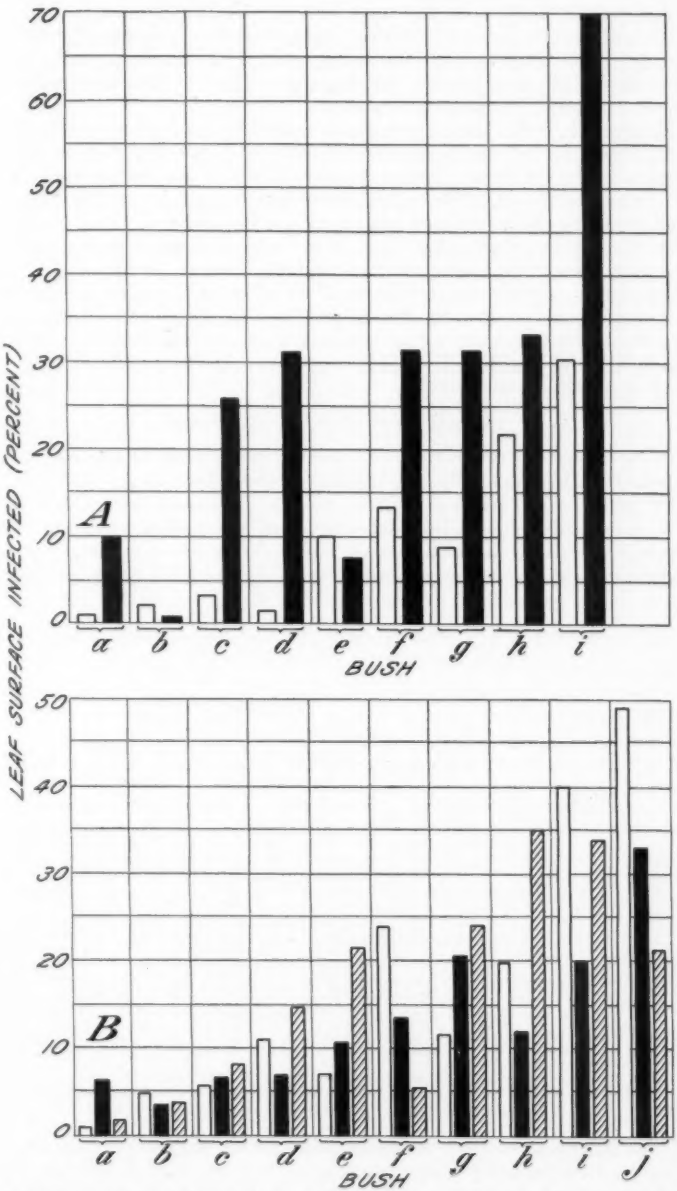

FIGURE 6.—Susceptibility of individual ribes bushes: *A*, *a–i*, open-form *R. inerme*: *B*, *a–j*, shade-form *R. viscosissimum*. 1925 infection, unshaded; 1927 infection, solid; 1928 infection, diagonally hatched.

While differences were far from being entirely consistent, in general a bush severely infected during one year also displayed relatively high susceptibility during the other years. Likewise a considerable proportion of the bushes exhibiting extreme relative resistance within a group did so consistently.

In general, *Ribes petiolare* was of uniformly high susceptibility and but little affected by weather conditions; however, the small proportion of the bushes of this species that were resistant were extremely and consistently so. The apparently low susceptibility of bushes *a*, *b*, and *c* in figure 5, *A*, and *a*, *b*, *c*, and *d* in figure 5, *B*, as compared with that of the remaining bushes in these groups, tends to confirm the evidence of the existence of a resistant strain or strains. In the other three species there are less definite indications of inherent intraspecific differences in susceptibility, and any such differences as may occur are apparently not so great as in *R. petiolare;* the available evidence (e. g., fig. 6, *B*) suggests, however, that even here they may be of appreciable magnitude.

DEVELOPMENT OF TELIA BY SPECIES

The proportions of the infected leaf surface which bore or for various reasons failed to bear telia are shown for the different species and their forms in table 7. *Ribes petiolare* and the shade and part-shade forms of *R. inerme* developed telia over most of the infected surface, while *R. lacustre* and the open form of *R. inerme* were much lower in the production of telia. *R. viscosissimum* was intermediate between the two types.

TABLE 7.—*Percentage of infected leaf surface which bore or failed to bear telia on inoculated Ribes, interior British Columbia, 1924–28*

Species	Infected leaf surface [1]—											
	Bearing telia			Bearing uredia but dead before producing telia			Lost through partial defoliation [2]			Necrotic		
	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
R. petiolare	89.8	98.7	94.0	6.0	0.7	1.5	3.0	0.4	4.2	0.6	0.1	[4] 0.2
R. inerme	35.2	84.0	81.3	24.9	5.7	8.3	34.2	7.8	8.2	5.0	2.2	2.0
R. viscosissimum	53.0	.....	63.5	29.4	.....	19.2	8.4	.....	5.6	7.3	.....	9.7
R. lacustre	11.8	16.3	35.8	79.8	80.2	46.6	3.2	0.3	4.4	5.1	2.8	10.0

[1] Basis, all plants tested, as in table 4.
[2] Includes natural defoliation as well as that induced by rust and drought before telia could be produced.
[3] Tested during 3 years only, 1926–28.
[4] In most cases insignificant percentages of the infected leaf surface still bore uredia at the time of final examination; consequently the percentages shown here usually total slightly under 100 for each form of each species.

Table 7 indicates that necrosis, i. e., death of infected leaf surface before either fruiting stage appears, was of comparatively little significance and that only in the case of open-grown *R. inerme* was falling of infected leaves from the bushes an appreciable factor in the reduction of telial sporulation. Most of the difference between infection and telial production is accounted for by death of leaf surface

bearing uredia. This occurred most commonly in the species and forms of relatively low telial production and was most striking in the case of *R. lacustre*, where it was apparently intensified by the warmth and dryness of midsummer. The great majority of the telia produced by this species developed at a relatively early date. Because of the partial loss of infected leaves in the natural cast of early-formed foliage and the failure of the rust to spread to other leaves, it is often difficult during the latter part of the growing season to find the rust on *R. lacustre*, even on bushes initially well infected.

Telial production per unit of leaf surface infected showed a general tendency in the present tests to increase with the percentage of leaf surface infected (table 8). This relationship was not sufficiently constant, however, to permit the certain prediction that because a species exhibits high susceptibility to infection it will have a similarly high percentage of the infected surface bearing telia, or vice versa. Variation in this relationship is evident between the species and forms shown. For example, the open form of *Ribes inerme*, with an average of nearly 14 percent of the leaf surface infected, bore telia on only about 35 percent of the infected surface, while the shade form of *R. viscosissimum*, with only 11.5 percent of the surface infected, bore telia on 63.5 percent of the infected surface. Similarly, there are wide discrepancies between production of telia on the various forms of *R. lacustre* and on the open form of *R. viscosissimum*, which falls in the same range as *R. lacustre* in percentage of leaf surface infected. Mielke and Hansbrough (*8*) found a far more extreme example of lack of correlation in the case of *R. roezli* (Reg.) Cov. and Brit., one of the most important California species. Tests of this species over a period of 2 years gave an average of about 46 percent of the leaf surface infected, while only about 0.7 percent of the leaf surface bore telia. In this case infection hastened defoliation of the test plants to such a degree that most of the infected leaves fell before telia could be produced. This species was tested outside its range, however, and may possibly show a considerably higher telial productivity in California.

TABLE 8.—*Percentage of leaf surface infected in relation to percentage of leaf surface bearing telia on inoculated Ribes, interior British Columbia, 1924–28*

Species and growth form	Leaf surface infected	Infected surface bearing telia	Species and growth form	Leaf surface infected	Infected surface bearing telia
	Percent	*Percent*		*Percent*	*Percent*
R. petiolare (part shade)	36.1	98.7	*R. viscosissimum* (shade)	11.5	63.5
R. petiolare (shade)	34.8	94.0	*R. lacustre* (shade)	8.1	35.8
R. inerme (shade)	23.0	81.3	*R. viscosissimum* (open)	6.6	53.0
R. petiolare (open)	22.6	89.8	*R. lacustre* (part shade)	4.3	16.3
R. inerme (part shade)	22.4	84.0	*R. lacustre* (open)	3.4	11.8
R. inerme (open)	13.9	35.2			

Thus, while there is a general tendency toward positive correlation between percentage of leaf surface infected and percentage of infected surface bearing telia, this correlation is by no means absolute. The leaf tissues of *Ribes petiolare* are comparatively thick and strong and, once infected, are generally capable of sustaining and permitting the growth of rust hyphae until a good crop of telia is produced. The shade and part-shade forms of *R. inerme* are also high in the percent-

age of infected surface bearing telia, but the open form is relatively low because of the susceptibility of its tissues to rust and drought injury. *R. viscosissimum*, while relatively low in susceptibility, is relatively high in the percentage of the infected surface bearing telia. The tissues of this plant are more resistant to rust and other injury than are the open form of *R. inerme* and all forms of *R. lacustre*. The latter species produces telia from relatively small proportions of the infected surface, although under favorable conditions the shade form and, to a lesser degree, the part-shade form sometimes show a good production of telia. The open form is characteristically meager in telial production.

TELIOSPORE PRODUCTON BY SPECIES

There are great differences between species in teliospore production per unit of leaf area bearing telia. In some cases telial columns are small and sparse, while in others they are comparatively large and so abundant as to be almost matted. The latter is frequently the case with *Ribes petiolare*, the infected surface of which characteristically produces a relatively dense stand of well-developed telial columns. The distribution of the columns is somewhat sparser on the infected surface of *R. inerme*, but the columns are frequently longer, particularly on the shade and part-shade forms. Very short stout columns are commonly noted on *R. viscosissimum*, and their distribution is usually more scattered than on *R. inerme*. Telial development on *R. lacustre* in nature is almost always relatively sparse, and the columns are generally small in diameter although often quite long. Data in table 4 are therefore unsatisfactory as indices of teliospore production.

Taylor (*18*) has made counts of telial columns and teliospores for several ribes species, including one (*Ribes lacustre*) involved in the present study. Her results, unfortunately, are not generally applicable, since the averages given were determined for units of total leaf surface rather than infected or telium-bearing surface. In the present study, relative values roughly expressing the difference in teliospore production per unit of leaf surface bearing telia were secured by ocular estimate of the average relative density and bulk of the telial columns on the telium-bearing surface for each ribes species and form. These estimates, based on comparative study of the extensive collections of representative specimens taken during the tests, and relative values indicative of the actual differences in teliospore production between the various species and forms, are given in table 9.

Other studies not yet reported have demonstrated that either *Ribes viscosissimum* or *R. lacustre* is quite capable of spreading enough infection under natural conditions to destroy associated pine. The enormous potential pine-damaging power of *R. petiolare* and *R. inerme* may therefore be readily inferred from the comparative ratings in table 9.

TABLE 9.—*Relative teliospore-production estimates for inoculated Ribes species, interior British Columbia, 1924–28*

Species	Form of species		
	Open	Part shade	Shade
RELATIVE PRODUCTION OF TELIOSPORES PER UNIT OF TELIUM-BEARING SURFACE [1]			
R. petiolare	100	85	75
R. inerme	60	70	80
R. viscosissimum	10		15
R. lacustre	5	6	7
RELATIVE TELIOSPORE PRODUCTION PER UNIT OF TOTAL LEAF SURFACE [2]			
R. petiolare	2,030	3,026	2,452
R. inerme	294	1,316	1,496
R. viscosissimum	35		109
R. lacustre	2	4	20

[1] Arbitrary scale: Relative mass of telial columns per unit of leaf surface bearing telia=100 in open-grown *R. petiolare*.

[2] The numbers given are relative values indicative of the actual differences in teliospore production between the various species and forms. The values were derived by multiplying average percentages of leaf surface bearing telia in table 4×100×the corresponding estimates shown in the upper part of table 9.

LOCAL AND ANNUAL DIFFERENCES IN INFECTION

The relative susceptibility and telial productivity of these four species and their forms in nature are fairly constant, and the average susceptibility of any one of them in Idaho seems to be about the same as in British Columbia despite some rather distinct climatic differences between the two regions. (See fig. 2, tables 4 and 5, and discussion.) There are, however, pronounced local and annual variations in the amount of infection in response to differences in the weather. An idea of the range of such variability may be gained from table 10, in which infection on groups of bushes in several different localities is shown for 2 or 3 consecutive years. In each locality the same groups of bushes were used during each year for which results are shown.

For the best initial infection from aeciospore inoculations in nature two conditions were found to be essential in the present tests: (1) That the leaves be young, which is their most susceptible stage (*6*), and (2) that favorable moist periods occur immediately following the inoculations. For uredial intensification thereafter a good distribution of favorable moist periods in synchronization with the production of urediospores is more important than the total amount of precipitation. Weather conditions during the 3 years differed widely in these respects.

Spring in 1926 was one of the earliest on record, stimulating an exceptionally early vegetative development of ribes. Favorable infection weather during the early part of the season, but mainly after the leaves had passed their most susceptible stages, was followed by severe drought which began in early June and continued into the latter half of August. In 1927 the season was late, with a corresponding retardation of the commencement of ribes leaf development. Early in the season moisture conditions were favorable, with precipitation above normal in May and early June. From then through July precipitation was somewhat below normal but well distributed, and there-

after it was considerably above normal as well as favorably distributed. Spring in 1928 was late, as in 1927, but most of May was relatively dry. Rains commenced in the latter part of May and from then into July precipitation was well above average and well distributed. This early-summer moist weather was succeeded by subnormal rainfall in August and by extreme drought during the remainder of the season. Monthly rainfall records covering the period from April to November at weather stations nearest the study areas are given for 1926 to 1928, inclusive, in table 11. These records have been compiled from daily weather reports which were used in the analysis of the above-mentioned infection conditions.

TABLE 10.—*Local and annual variation in severity of infection of Ribes species, British Columbia, 1926–28*

Growth form, species [1] and locality	Total leaf surface [2]—					
	Infected			Producing telia		
	1926	1927	1928	1926	1927	1928
Open form:						
R. petiolare:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Kelowna swamp	27.9	40.2	----	27.8	36.7	----
Nine Mile Creek	51.4	35.0	3.0	51.0	34.3	3.0
R. inerme:						
Kelowna swamp	1.9	44.6	----	1.9	14.7	----
Nine Mile Creek	5.5	21.4	10.0	3.4	1.8	3.6
R. viscosissimum:						
Canyon Creek	.6	12.4	17.1	.2	3.3	4.7
Camp McKinney	----	7.5	.7	----	6.5	.4
R. lacustre:						
Canyon Creek	.05	13.6	3.9	Trace	.8	.5
Nine Mile Creek	.9	4.9	.7	.1	.4	.2
Camp McKinney	----	12.6	8.7	----	1.4	1.8
Shade form:						
R. petiolare:						
Kelowna swamp	.03	1.5	----	.03	1.4	----
Summerland	30.0	46.6	----	29.8	38.0	----
Haynes Creek	20.7	37.0	----	20.5	35.3	----
Nine Mile Creek	46.5	36.8	20.0	46.3	36.4	19.6
Camp McKinney	----	65.7	71.3	----	63.0	65.4
R. inerme:						
Kelowna swamp	21.1	34.4	----	21.1	30.1	----
Nine Mile Creek	18.7	29.1	10.4	17.3	20.5	8.2
R. viscosissimum:						
Canyon Creek	.9	11.8	22.9	.3	4.7	6.9
Camp McKinney	----	12.3	10.9	----	11.4	7.5
R. lacustre:						
Kelowna swamp	.01	7.2	----	Trace	3.5	----
Canyon Creek	.1	19.8	4.6	Trace	2.3	.6
Haynes Creek	2.1	13.8	----	.4	6.4	----
Nine Mile Creek	7.5	10.2	1.0	1.1	3.4	.3
Camp McKinney	----	13.3	11.2	----	8.9	3.4

[1] The number of bushes used as a basis in each test ranged from 16 to 64 but was usually about 50.
[2] Leaders indicate that the group was not tested.

Weather at the time of inoculation in 1926 was favorable to infection, but ribes leaves were too far advanced (*6*) for best results except at the Nine Mile Creek and Haynes Creek areas. In these two localities the development of vegetation was 7 to 10 days behind that at lower elevations and ribes leaves had not passed beyond the highly susceptible stage at the time of inoculation. Midseason intensification was limited by the decidedly subnormal rainfall during June and July.

TABLE 11.—*Rainfall* [1] *near study areas during growing season, interior British Columbia, 1926–28*

Locality and year	April	May	June	July	August	September	October	November
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
Kelowna:								
1926	0.58	1.13	0.33	0.20	1.20	0.96	1.08	1.49
1927	.23	.81	.96	.33	1.97	2.71	1.76	2.95
1928	1.34	.82	1.86	.64	.82	.10	.49	.57
Normal	.73	.79	1.03	.56	.84	1.05	1.12	1.14
Summerland:								
1926	.83	.98	.69	.16	.83	.55	.73	.72
1927	.28	.82	.90	.54	1.37	2.17	1.34	2.57
1928	1.57	1.16	1.48	1.65	.23	.01	.39	.35
Normal	.72	.75	1.03	.51	.69	.78	.89	1.02
Rock Creek:								
1926	.76	1.31	1.53	.25	2.16	2.15	1.44	1.54
1927	.59	1.62	1.93	.50	1.25	4.06	1.22	2.61
1928	1.40	.40	3.76	1.73	.52	.00	.42	.42
Normal	1.00	1.18	1.83	.98	.96	1.09	.89	1.19
Oliver:								
1926	.33	.48	.49	.24	1.43	1.36	.94	.85
1927	.23	.77	1.55	.20	.96	1.62	.73	1.59
1928	1.00	.58	1.31	1.00	.28	.00	.23	.50
Normal	.43	.45	.97	.26	.43	.68	.67	.90

[1] From British Columbia Department of Agriculture weather reports.

The season of 1927 was outstandingly favorable for the development of the rust over most of the region. A good synchronization of the early susceptible stages in ribes leaf development and suitable weather generally attended the inoculations, resulting in good initial infection, and the well-distributed precipitation that followed encouraged an abundant uredial intensification. Mild rains from May 17 to June 13 stimulated the development of the rust on *Ribes lacustre* particularly, since any decided increase of infection in this species is limited, by the nature of its susceptibility, to the early part of the season. The only test groups on which infection was less severe than in 1926 were at Nine Mile Creek, where both open and shade forms of *R. petiolare* showed relatively less rust in 1927. Growth starts several days later in this species than in associated ribes, and by the middle of May, at the time of the first inoculations in 1927, only a few small leaves had appeared on the Nine Mile Creek bushes. A second inoculation in early June was not immediately followed by rain, and the initial infection resulting from the inoculations was therefore relatively light. Although infection intensified well later in the season, it did not entirely overcome its original handicap on these plants. *R. inerme* is very sensitive to weather conditions, and the open form of this species suffered so much defoliation during a local midseason heat wave at Nine Mile Creek that very little infected surface was left to produce telia.

In 1928 a considerable wait was required before weather favorable for inoculations arrived, and by that time, in late May and early June, the leaves had generally passed their most susceptible stages except at Camp McKinney. The plants at Canyon Creek were inoculated three times, and although many of the leaves were maturing and becoming resistant, infection was facilitated by showers following two of these inoculations. The rust developed particularly well there on *Ribes viscosissimum*. In this species, age of leaves at time of initial infection exercises less influence than in any of the other three ribes species (*6*). *R. lacustre*, the only other species tested

there, showed a decided reduction in degree of infection compared with that which it developed in 1927. Leaves of all the ribes were still fairly susceptible at Camp McKinney, but were becoming resistant at Nine Mile Creek, and therefore relatively little infection developed at the latter place in spite of favorable weather conditions following two inoculations. The prolonged dry spell beginning in late summer reduced subsequent intensification over the entire region, having an exceptional effect in the case of open-grown *R. petiolare* at Nine Mile Creek, where leaves, normally susceptible throughout their life, became leathery and resistant. Because of the importance of late pyramiding of infection in this species, the drought exercised an extremely retarding influence in this case.

Another group which exhibited an extraordinarily wide reduction from 1927 levels at Nine Mile Creek was the shade form of *Ribes lacustre*. By the time of the inoculations here in 1928, the leaves of this form had hardened off to a stage of such resistance that there was comparatively little initial infection or subsequent intensification.

Still another group to show a reduction in infection from 1927 levels was the open form of *Ribes viscosissimum* at Camp McKinney. This was surprising in view of the fact that the open form of *R. lacustre* there showed but a relatively slight reduction. Initial infection was lighter on both groups than in 1927, but a good wave of intensification raised the percentage of surface infected on the *R. lacustre* while the rust on *R. viscosissimum* remained practically at a standstill.

Considering the species individually (table 10), *Ribes petiolare*, with the exception of the open form at Nine Mile Creek in 1928, showed the smallest percentage of seasonal variation in the degree of infection. In this case the resistance was clearly induced by an extraordinary effect of the weather on the host. The shade-form plants of *R. petiolare* at the Kelowna swamp showed high resistance in both 1926 and 1927, while the open-form plants were heavily infected in both years. Here the resistance appeared to be primarily inherent, for conditions for infection in the shade at this place were favorable in both years, as indicated by the reaction of the shade-form plants of *R. inerme* nearby. *R. petiolare* as a rule maintained a high degree of susceptibility and was consistently high in the proportion of its infected surface that bore telia. This was true whether resistance appeared inherent, as in the case of the shade form at the Kelowna swamp, or induced, as in the case of the open form at Nine Mile Creek in 1928. Observations of the part-shade form of this species, aside from the results under immediate consideration, indicate that its reactions are similar to those of the shade form.

The shade form of *Ribes inerme* was generally similar to that of *R. petiolare* in uniformity of local and seasonal infection and in the ratio of the percentage of surface bearing telia to the percentage of surface infected. At the same time its open form in the same places and years was highly variable in both respects (table 10). The very light infection of this form at the Kelowna swamp in 1926, as compared with the heavy infection there under the more favorable weather conditions of 1927, and the low proportion of infected leaf surface bearing telia on the Nine Mile Creek plants in 1927, as compared with the relatively high proportion bearing telia on these plants under the lighter infection conditions of 1926, clearly illustrate this

point. As already shown, the frequent failure of this form to bear telia abundantly under conditions of heavy infection is caused primarily by its susceptibility to injury and defoliation from rust and drought before telia can be produced. The foregoing conclusions for this species, in addition to being supported by all results in the present tests, were further substantiated by observation of its behavior at two centers of heavy natural infection in north Idaho. The part-shade form generally resembles the shade form in its reactions.

The data for both forms of *Ribes lacustre* and *R. viscosissimum* in table 10, with the possible exception of the results at Camp McKinney for the shade and open forms of *R. lacustre* and the shade form of *R. viscosissimum*, indicate a high degree of variability in seasonal reaction to the rust within a relatively limited range of susceptibility. As a rule these ribes species show a considerable degree of variation in their group reaction from season to season both in percentage of leaf surface infected and percentage of infected surface bearing telia. In other words, they may vary from almost immune in an unfavorable season to moderately susceptible when conditions are especially favorable for rust development. Of the two, *R. viscosissimum* appears the more dangerous as a potential source of infection of white pines, at least as far as ability to produce telia is concerned.

Elevation is a local environmental factor which has an influence on the phenology of both the rust and the host. The short growing seasons at high elevations provide a more limited period for uredial intensification. At such elevations, however, the greater and more frequent precipitation may create more opportunities for infection, thus resulting in a greater abundance of rust on ribes at higher than at lower elevations, particularly in a dry season.

Another environmental factor of some local importance in variation in the development of rust is the occurrence of dew. At the Kelowna swamp and to a lesser degree at Canyon and Nine Mile Creeks there was evidence that heavy dews caused uredial intensification. This was also observed along the bottoms of many of the moist canyons in the white pine belt of north Idaho. Intensification from dews is generally much less than that caused by rains.

From the foregoing discussion it is evident that weather and local environmental conditions influence the development of the rust on ribes directly and by affecting the host. The important effect of host phenology upon initial infection and subsequent intensification has been mentioned. For the best initial infection it is essential that the period in which ribes leaves are in their most susceptible stages of development be synchronized with the period of maximum aeciospore production and with moisture conditions favorable for infection. To assure the maximum intensification thereafter, moist periods favorable for infection should come at frequent intervals coinciding with periods of abundant urediospore production. Normal irregularities in weather during the season almost invariably prevent any such ideal coincidence of favorable factors, although such conditions may occasionally be approached in one locality or another. In this respect climate is perhaps primarily important in its influence on the rust. On the other hand, abnormally cold or hot spells or other unseasonable weather conditions at any time during the growing season may and frequently do completely upset the processes of infection and inten-

sification, primarily through their influence on the host. Thus, to use an extreme example, the development of ribes leaves and aeciospores may be stimulated by early-season warm weather, rains may follow and good infection take place, and then the plants may be locally or generally defoliated by frost or drought.

To summarize, *Ribes petiolare*, disregarding its resistant form, which probably does not represent more than 10 to 15 percent of the species, and disregarding exceptional cases where a rather high degree of resistance was encountered, appears to be the most uniform of all species in its local and seasonal reaction to the rust. In general, it ranges from moderately to very highly susceptible and produces telia abundantly from the infected surface. The shade form of *R. inerme* exhibits similar uniformity but is less susceptible and considerably lower in telial production. The open form of this species shows considerable differences in amount of infection and telial production from one year to another. This variation is apparently caused by sensitiveness of this form to abnormal temperatures and drought. *R. lacustre* and *R. viscosissimum* are generally resistant, but in their more susceptible forms, and particularly in the case of *R. viscosissimum*, may be moderately susceptible when weather conditions are favorable.

GENERAL RESULTS

The results of the study indicate a susceptibility and capacity for telial production in *Ribes petiolare* approaching that of the cultivated black currant from plantings of which damaging infection has been known to spread to pines for over a mile. *R. inerme* compared favorably with other highly susceptible wild species from concentrations of which instances of similar spread have been observed. Even the relatively low telial production of *R. viscosissimum* and *R. lacustre* is known to be sufficient under moderately favorable conditions to seriously damage associated pines. The studies have given results of basic value in devising scouting programs to determine the extent of spread of the rust,[8] and in the development of control plans (*20*).

SUMMARY

This paper reports the results of studies of the white-pine blister rust in British Columbia and Idaho. The investigations were undertaken to determine the susceptibility and telium-producing capacity of the four principal ribes species in the commercial range of western white pine (northern Idaho, northeastern Washington, and western Montana), as a preliminary measure of the potential pine-infecting power of these species.

Since the tests were concerned entirely with the probable comparative importance of these ribes species in spreading infection to pines, methods used in other rust investigations were not applicable. New systems of recording data, developed to meet the needs of the study, are fully described.

The four species studied were *Ribes petiolare*, *R. inerme*, *R. viscosissimum*, and *R. lacustre*, all of which were present in the range of the rust in British Columbia. The studies were conducted under

[8] BLISTER RUST IN THE FAR WEST. Jan. 1 to Dec. 31, 1928. Spokane Branch Off. Blister Rust Control. 379 pp. [Typewritten report] See pp. 178–188.

natural conditions and included 5,098 tests (nearly 3,000,000 leaves on 2,740 different bushes) in interior British Columbia, from 1924 to 1928, inclusive, and 1,358 tests (nearly 500,000 leaves) in the coastal region of British Columbia. Results were substantiated by 562 tests (nearly 400,000 leaves) conducted in the white pine region of northern Idaho during 1933 and 1934. Ribes species other than those studied are seldom encountered in the commercial range of western white pine.

Ribes petiolare was found to be extremely susceptible, approaching cultivated black currant in both severity of infection and production of telia. *R. inerme* is also highly susceptible, under very favorable conditions, equaling or surpassing *R. petiolare* in extent of infection, and bears abundant telia when growing in the shade. *R. viscosissimum* and *R. lacustre* are more resistant. The latter occasionally becomes moderately infected, but is almost always quite low in production of telia. When density and size of telial columns are considered, the relatively greater teliospore production and potential danger to associated pines of *R. petiolare* and *R. inerme* appear even more pronounced. Infection is heavier and telia are more abundant on plants in the shade than in the open. Data on average infection, telium production, and teliospore production are given for open, shade, and part-shade forms of each species.

Relative susceptibility and ratios of telium production to percentages of leaf surface infected remained fairly constant, but weather and other environmental factors caused pronounced local and annual differences in the degree of infection between individuals and groups of individuals of the same species and form. It also appeared that variation was affected by the inherent differences in susceptibility between individuals.

Results of the studies have been of basic value in formulating plans for control of the rust in northern Idaho and adjacent Montana and Washington.

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A MICROCHEMICAL COLORIMETRIC pH PROCEDURE FOR DIFFERENTIATING THE TELIA OF CRONARTIUM RIBICOLA AND C. OCCIDENTALE[1]

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INTRODUCTION

Efficient control of the white-pine blister rust (*Cronartium ribicola* Fisch.) is dependent upon early recognition of advance infections on ribes. As the distance between the areas in the West infected respectively with *C. ribicola* and with *C. occidentale* Hedgc., Beth., and Hunt grows shorter, the differentiation of these two rusts in all stages becomes increasingly necessary. The present work was undertaken because there was no known method of differentiating the two rusts in the telial stage. Very frequently from August to the end of the season the ribes leaves collected bear telia only. It was highly desirable that the differentiation be a quick, 100-percent effective method, applicable to small and sparsely infected specimens such as advance infections are likely to furnish. All the standard biological stains that had been tried failed to give any differentiation. Measurements of length and width of the telia of the two species gave unsatisfactory data, showing definite differences between the averages of a considerable number of specimens, but overlapping and therefore of no significance when applied to one unknown sample.

It was assumed that certain constant differences in morphology, physiology, and behavior occurring in the other spore stages of these two species were concurrent with some basic physical-chemical difference in the telial stage, however small or variable. Because pH measuring methods indicate by color very minute differences in acidity or alkalinity, which can have marked influence on the physical-chemical properties and reactions of tissue constituents, a series of exploratory studies of pH reactions was made.

RESULTS OF EXPLORATORY EXPERIMENTS

Though the exploratory studies involved different methods of approach and were constantly refined and changed, from the very beginning all the findings were consistent and supported the theory of the reactions occurring in this procedure.

The results of these preliminary studies showed some chemical difference in the two species as indicated by their action upon various indicators, the effects of indicators on them, and differences in the pH values of measured quantities of water in which weighed amounts of telia were extracted. In each case, however, the differences were

[1] Received for publication Feb. 16, 1937; issued September 1937. This work, which was done at the Bureau of Standards, U. S. Department of Commerce, is one of a series of cooperative investigations carried on by that Bureau and the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. The research was supported in part by Emergency Conservation Work.

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those between the averages for a number of telia, and overlapped in much the same way as spore measurements. They were consistent for not over 70 percent of the specimens tested and negative or without significance for the others, or else very slight or transitory. For instance, some indicator solutions or mixtures containing indicator solutions produced colors in the two species differing in hue as much as two indicator solutions 0.1 to 0.5 pH apart. It was, however, impossible to establish color standards with any of the methods developed in these preliminary experiments because the initial color of the telia of both species is yellowish, ranging from light honey to reddish brown, and varies in hue and intensity more within a single species than an indicator varies in hue and intensity over 0.4 to 0.5 Hp. Furthermore the phenolsulfonphthalein indicators, the precise application of which to pH problems has been developed [3] [4] by S. F. Acree and his coworkers are yellow on the acid side and intensely red, green, or blue on the alkaline side of their range. Obviously the combination of the variable initial color of the telia with the color of the indicator results in color massing and proportionate variation in the final color, and only a procedure resulting in two distinct colors, separated on the spectrum, would supply unmistakable differentiation of these two species. As pointed out previously, it was also necessary to have a method applicable to both sparsely and heavily infected specimens.

EQUIPMENT AND PROCEDURE

A procedure was finally developed that caused the telia of *Cronartium occidentale* to appear a bright green and those of *C. ribicola* a brilliant blue, when viewed under the compound comparison microscope or the compound microscope with a magnification of 108 ×. Citations to color charts for a more accurate description of these colors are given along with other details of the procedure.

The procedure consists essentially in consecutive measured exposure of the telia to 0.1 N hydrochloric acid, distilled water, and 0.001 M bromphenol blue adjusted to pH 7.6 (three units above the pH color range (3.0–4.6) of this indicator). Exposure to the acid and water is measured by the time interval of and the amount of agitation during each step. Exposure to the indicator is controlled by regulating the thickness of the film of indicator in the mount. The exploratory experiments gave evidence of a minute but not easily demonstrated physical-chemical difference between the two species. The procedure intensifies and amplifies this difference and expresses it in two definite and unmistakable colors.

The critical factors in the procedure seem to be the balance between the exposure of the telia to the hydrochloric acid and to the water and the choice of indicator and its pH adjustment. If the concentration of the acid solution, the length of the time intervals in acid or water, or the pH value of the bromphenol blue are changed at random, the results vary over a wide range of yellow, brown, green, blue, purple, and mixtures of all or any of these colors, or the two species

[3] BIRGE, R. T., and ACREE, S. F. ON THE QUINONE PHENOLATE THEORY OF INDICATORS. A SPECTROPHOTOMETRIC METHOD FOR MEASURING THE CONCENTRATIONS OF QUINOIDAL AND LACTOIDAL SALTS AND THE EQUILIBRIUM AND AFFINITY CONSTANTS OF THE PHENOLPHTHALEINS AND PHENOLSULFONPHTHALEINS. Jour. Amer. Chem. Soc. 41: 1031–1050. 1919. (Contains references to earlier papers.)

[4] ACREE, S. F., and FAWCETT, E. H. THE PROBLEM OF DILUTION IN COLORIMETRIC H-ION-MEASUREMENTS. II—USE OF ISOHYDRIC INDICATORS AND SUPERPURE WATER FOR ACCURATE MEASUREMENT OF HYDROGEN-ION CONCENTRATIONS AND SALT ERRORS. Indus. and Engin. Chem. Analyt. Ed. 2: 78–85, illus. 1930. (Contains references to papers appearing after 1919.)

may stain the same shade of green or blue, different shades of green or blue, or both green and blue in the same mount. It is probable that variations in the exposures and concentrations used in this procedure could be rearranged so that the balance would not be destroyed and the results would be the same.

The success of this procedure indicates that a combination of microchemical technique and pH procedure may offer new possibilities for the differentiation by color of species and varieties otherwise indistinguishable. There is a definite need for such methods in the study of such groups of fungi as the rusts, where the lack of any known method of artificial cultivation deprives the investigator of this prolific source of criteria for species identification.

For readers who may be concerned with solving a similar problem, the procedure will be described as it was carried out during the development of the process and will, therefore, include the equipment and handling necessary for putting two preparations through the same treatment, separately and as nearly simultaneously as possible. Any discrepancies in equal processing of the two samples, due to the necessity of starting one first, were kept at a minimum by starting one sample first in each separate step of the process and then repeating and starting the other sample first each time.

The following items were assembled ready for use:

(1) A dissecting microscope; wide-angle lenses greatly increase comfort and efficiency.
(2) A comparison microscope equipped with a 9× ocular and 16-, 8-, 4-, and 2-mm objectives, but for these experiments the 16-mm objective would have been sufficient.
(3) Samples to be tested: Pieces of ribes leaves one-eighth to three-eighths of an inch across bearing some normal fully developed telia.
(4) A clean, highly polished curved scalpel with square end and convex cutting edge.
(5) A clean, polished, slender-tipped pair of forceps.
(6) A small water-color camel's-hair brush with the bristles cut off straight across the thickest part of the brush.
(7) A bottle of 0.1 N hydrochloric acid.
(8) A beaker of 100-cc capacity, containing 50 cc of distilled water.
(9) Four glass jars or flasks with tops or stoppers of about 30-cc capacity, each containing 25 cc of distilled water; two jars to be used for first washing and two for second washing.
(10) A supply of small torn pieces of absolutely clean dry filter paper.
(11) Some chemically clean cover glasses and slides, with labels. The labels must be pasted on without smearing excess glue out onto the slide, to avoid chemical contamination.
(12) Wash bottle or beaker containing distilled water.
(13) A waste jar or beaker.
(14) Two watch glasses about 2 inches in diameter, each containing 1 cc of 0.1 N hydrochloric acid. This acid should be put in the watch glasses just before starting the test.
(15) 0.001M bromphenol blue adjusted to pH 7.6, preferably in a pyrex dropping bottle.
(16) Some sterile surgeons' gauze.

To cut down the time of manipulation and so keep the processing of the samples of the two species as nearly simultaneous as possible, the same camel's-hair brush, forceps, and scalpel were used in each step and for both species. After each single manipulation, however, the instrument used was washed in distilled water and dried on sterile surgeon's gauze. The possibility of introducing error-producing chemicals through contact with the gauze was checked by previously boiling the gauze in distilled water for 10 minutes and comparing the

pH reading of this water with that of the same quantity of distilled water from the same source, boiled 10 minutes. The gauze produced no change in the pH value of the water.

The procedure is as follows:

(1) Note the starting time and drop the samples of leaves, with telia-bearing surfaces down, into watch glasses containing 2 cc of 0.1 N hydrochloric acid.

(2) Under the dissecting microscope, reverse the samples so that the telia are up and with the camel's-hair brush tease air bubbles from the telia and leaf surfaces; turn back so that the telia-bearing surfaces are down; agitate by giving the watch glasses two left-to-right motions with the hand.

(3) Twenty minutes after starting time, agitate again by two left-to-right motions with the hand.

(4) Twenty-seven minutes after starting time, agitate again.

(5) Thirty minutes after starting time, with forceps remove the leaf samples from the acid, touch with filter paper, pass through the water in the 100-cc beaker, touch with filter paper to remove excess water, and put into the jars containing the first wash water and agitate.

(6) Forty-five minutes after starting time, agitate the jars containing the samples and first wash waters, and with forceps transfer the samples to the second wash water and agitate.

(7) Sixty minutes after starting time, agitate the second wash waters containing the samples, remove the samples with forceps to the slides and place them to one side of mounting spaces on slides that are already in focus under the dissecting microscope.

(8) Draw excess water from the samples with filter paper but leave enough to keep the telia thoroughly wet.

(9) Putting the scalpel close to the bases of the telia, lift or drag them from the leaf. When about a dozen are removed to each slide drag them to the centers of the areas intended for mounts, remove excess water with filter paper, put enough bromphenol blue, pH 7.6, on each lot of telia to make a film between the slide and cover glass about the same thickness as the thickness of the telia, and put on the cover glasses.

(10) Remove the slides at once to the comparison microscope for observation. During the process of removing the telia from the leaf samples and putting on the bromphenol blue, pH 7.6, the telia must stay wet, but excess pools of water must be avoided. Obviously an excess of water would affect the indicator. Under the comparison microscope it will be observed that the indicator immediately surrounding the telia of both species at first turns yellow. This yellow slowly diffuses into the indicator of the remainder of the mount, and more or less rapidly the telia of *Cronartium ribicola* become blue and those of *C. occidentale* green. This takes from 5 to 15 minutes.

(11) When the blue or green of the telia approaches its maximum brilliancy, place the slides under the dissecting microscope and with filter paper draw off all the indicator possible. This must be done quickly and be so manipulated that all is drawn from around the telia at least, so that any small remaining amount must not contact the telia. Press the cover glass firmly down upon the telia and slide by applying weights or wooden pinch-type clothespins. This must be done so that the mount is as nearly as possible the thickness of the telia but mashing of the latter should be avoided. A little experience

is needed to develop judgment as to when to draw off the indicator to secure brilliant but not overstained preparations and how much pressure will make a thin mount without crushing the telia.

Staining with the residual indicator is evidently greatly intensified by the evaporation of the water and proportionate increase in the concentration of the indicator. This can be easily and quickly controlled by running paraffin oil under the cover glass as soon as the colors attain the desired intensity. This makes a clearer, more transparent preparation, but the mount is too messy and dust-collecting for anything but temporary use.

Understaining results in softer, less true blues and greens; overstaining, in bluish or greenish black. The mashed telia are always paler and sometimes a less true blue or green. This may be due to the smaller mass of color in a thinner layer of cells or to some chemical reaction that occurs when the tissue is crushed. It is not necessary to leave the clothespins on more than 20 to 30 minutes, but no harm is done if they remain on overnight. After the clothespins or weights are removed, these preparations can be stored away from the dust and kept for several months without ringing with balsam, but it is necessary to handle them carefully to prevent the cover glass from slipping off. For this reason slides that are to be kept should be ringed with Canada balsam or collodion too thick to run under the cover glass. To facilitate ringing, the balsam can be warmed; but if it contacts the telia, their color is sometimes changed. Preparations sealed with balsam or collodion retain their transparency and full differential color values for at least a year.

Oil mounts can also be made by removing the clothespins or weights and the cover glass, adding a drop of paraffin oil, replacing the cover glass and ringing the mount with Shuh's cement. Very clear slides that will keep from 6 to 10 weeks are so obtained. In time, however, the colors lose their brilliancy.

As stated previously, the differential characteristic of this process is that *Cronartium ribicola* stains blue and *C. occidentale* green. The blue will vary from a brilliant deep hue to a duller or paler blue and the green from a deep bluish green to a softer grayer or pale green. Not all the telia on a slide stain evenly; some are deeper or paler, harder or softer, more or less true blue or green, but *C. ribicola* appears definitely blue and *C. occidentale* definitely green, under a magnification of 108 ×. For accurate description of these colors, Ridgway's [5] Color Standards and Maerz and Paul's [6] Dictionary of Color were consulted. Because of the transparent quality of the colors in the latter, both the greens and blues were best matched in it. The blues corresponding most closely to the blues in the *C. ribicola* telia are found in Maerz and Paul's plate 34 L 7–12, and the greens matching those in *C. occidentale* best are found in their plate 30 L 6–12. Of Ridgway's colors, the blues matching best are those in plate VIII, light cerulean blue, cerulean blue, oxide, and Antwerp blue, and the greens matching best are those in plate VII, light blue, Guinea, and dark viridian green, and plate XVIII, oriental and dark yellowish green.

Upon further study with a magnification of 160 × or more, it is evident that the blue telia of *Cronartium ribicola* are made up of

[5] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.
[6] MAERZ, A., and PAUL, M. R. A DICTIONARY OF COLOR. 207 pp., illus. New York. 1930.

white matrix or cell wall and deep-blue spores or spore contents with sometimes a little very pale yellow matrix at the tip. Those of *C. occidentale* have a yellow or pale yellow-green matrix, green or green-blue spores or spore content, with sometimes a few scattered patches of blue where the indicator has evaporated on the telia surfaces. The blue when present is not the clear, brilliant, and undiluted blue of the *C. ribicola* telia, but dull and greenish. The bright-green color seen under the lesser magnification is apparently the composite color of the layers of pale and deep green, yellow and green, yellow and blue, green or green blue. For differentiation purposes a magnification of 108 × or less is best, because it shows clearly and definitely blue telia if the specimen is *C. ribicola* and green if it is *C. occidentale*. The reliability of this procedure for identification was tested on 173 specimens of known origin.

The development of this procedure involved a great deal of experimentation in precise technique which was greatly facilitated by the use of the dissecting and comparison microscopes. The dissecting microscope assists in the elimination of such sources of inaccuracy as air bubbles on the telia and leaf surfaces in the acid bath, and excess water in the mount. The simultaneous observation of the color changes in the two mounts, made possible by the use of the comparison microscope, saves innumerable timing and time records, and obviates the necessity of depending upon the memory for color comparisons.

SUMMARY

The established methods of species identification failed to furnish means of differentiating *Cronartium ribicola* and *C. occidentale* in the telial stage.

It was assumed that certain constant differences in the morphology, physiology, and behavior occurring in the other spore stages would be concurrent with some slight but basic physical-chemical difference in these two species that would occur also in the telial stage. Studies involving pH reactions were made because of the minute differences that such methods reveal and with the hope that if such a difference existed it could be demonstrated unmistakably by color.

Many exploratory experiments showed that there was a definite and consistent but not easily demonstrated microchemical difference between the two species. The objective then became the development of a treatment to amplify this difference and an indicator solution to demonstrate it. This was accomplished by certain treatments with dilute acid, distilled water, and bromphenol blue under pH control.

The results of this work indicate the opening of a new field of study for the solution of similar problems. The value of applying pH methods to problems involving study of very subtle biological differences lies in the delicacy and precision of the reactions. This is evidenced by the consistency of the results when different indicators were used in various ways and by the variable results caused by slight alterations in the procedure.

MERMIS SUBNIGRESCENS, A NEMATODE PARASITE OF GRASSHOPPERS[1]

By J. R. CHRISTIE[2]

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INTRODUCTION

In a previous paper [3] the author pointed out that in the United States the most common nematode parasites of grasshoppers are *Agamermis decaudata* Cobb, Steiner, and Christie, 1923, and *Mermis subnigrescens* Cobb, 1926,[4] species belonging to the family Mermithidae. The life history and the economic significance of the former species were discussed. The present paper deals with *M. subnigrescens*.

Whereas *Agamermis decaudata* is occasionally found in insects other than grasshoppers, *Mermis subnigrescens* appears to be strictly a parasite of grasshoppers. It has been found naturally infesting nine different species of these insects, including both Tettigoniidae and Acrididae; two additional species have been experimentally infested. It has not been found in crickets, even though they were collected in fields where a large percentage of the grasshoppers harbored the parasite. Numerous attempts to experimentally infest other insects, including crickets and mole crickets (Gryllotalpinae), have not been successful.

Mermis subnigrescens occurs throughout the New England States and westward to Minnesota, Iowa, and Missouri. Its range apparently does not extend as far south as that of *Agamermis decaudata*, for it is rarely found in the vicinity of Washington, D. C. The present paper is based on investigations conducted largely at Woods Hole, Mass., and statements regarding life history and behavior apply to that locality.

LIFE CYCLE

The life cycle of *Mermis subnigrescens* differs in one important respect from that of *Agamermis decaudata*. Eggs of the latter species are deposited in the soil. Upon hatching, the larvae migrate to the surface, climb the vegetation, and seek newly hatched grasshopper nymphs, which they enter by boring through the body wall. Eggs of *M. subnigrescens* are never deposited in the soil. Gravid females migrate to the surface and climb the vegetation, on which they deposit their eggs. Grasshoppers, while feeding, swallow the eggs, which on reaching the alimentary tract promptly hatch. The larvae penetrate the wall of the alimentary tract and enter the body cavity, where they develop.

[1] Received for publication Feb. 16, 1937; issued September 1937.

[2] The author wishes to acknowledge the aid of W. D. Courtney, who assisted with field work during the summer of 1929; of B. G. Chitwood, who assisted during the summer of 1930; and of Gerald Thorne, who assisted during the summer of 1931. Figures 1, *B*, and 3 were taken from unpublished notes of the late N. A. Cobb.

[3] CHRISTIE, J. R. LIFE HISTORY OF AGAMERMIS DECAUDATA, A NEMATODE PARASITE OF GRASSHOPPERS AND OTHER INSECTS. Jour. Agr. Research 52: 161–198, illus. 1936.

[4] COBB, N. A. THE SPECIES OF MERMIS, A GROUP OF VERY REMARKABLE NEMAS INFESTING INSECTS. Jour. Parasitol. 13: [66]–72, illus. 1926.

THE EGG

The egg (figs. 1, *A*, and 2) is protected by two coverings, the outer of which is easily removed. With this outer covering removed, the egg (fig. 1, *B*) has the shape of a sphere slightly compressed at the poles.

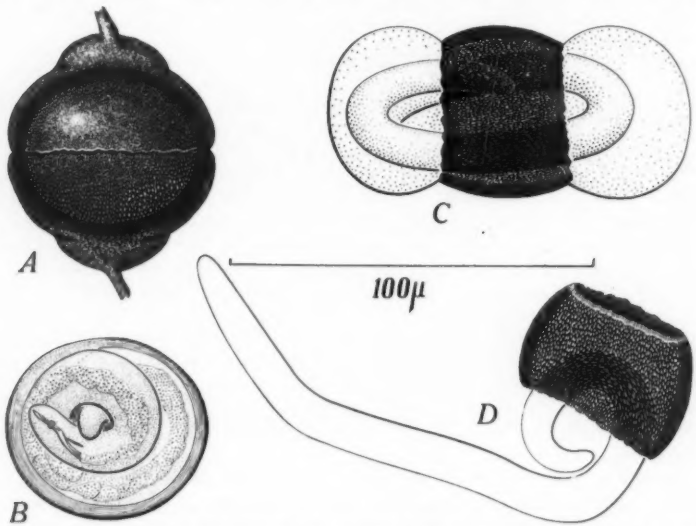

FIGURE 1.—Egg of *Mermis subnigrescens: A*, Showing outer and inner layers of shell; *B*, with outer layer of shell removed, showing larva within; *C*, in process of hatching; *D*, with larva emerging.

The diameter from pole to pole is 50μ to 54μ and at the equator 53μ to 56μ. The inner covering or shell is about 2.5μ in thickness and brown in color. The outer covering is divided into two cuplike

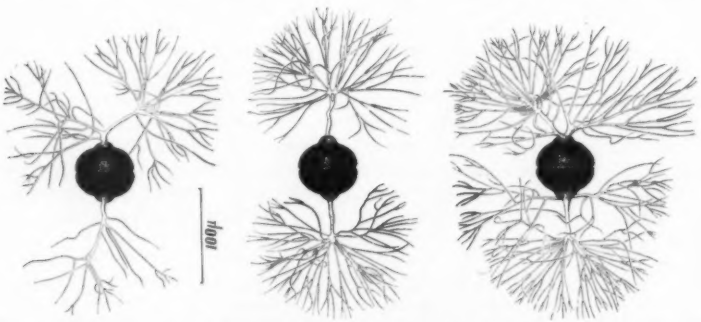

FIGURE 2.—Eggs of *Mermis subnigrescens* showing variations in the form of the byssus. (Drawn from photomicrographs.)

parts by a groove at the equator, and at each pole there is a raised or thickened area formed by the attachment of the entangling appendages or byssi. The size and shape of these polar thickenings vary considerably, as does also the form of the byssus (fig. 2). There are

two opposite areas at the equator where the color is lighter than elsewhere, and these areas are partially dissolved by the digestive fluids of the host, permitting the escape of the larva. At the time of deposition the egg contains an infective larva that has molted at least once (fig. 1, *B*).

The time of maximum egg deposition is usually during June and July, but, as will be discussed later, this is controlled by weather conditions. On foliage an egg remains viable throughout the summer, the larva presumably being protected from the sun's rays by the brown pigment in the shell. Eggs kept in water in a corked bottle at room temperature remain viable for at least 2 months; when kept on slightly moistened filter paper in a moist chamber some remain viable for a year.

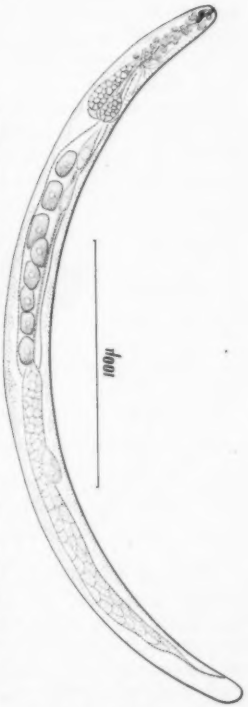

FIGURE 3.—Recently hatched larva of *Mermis subnigrescens*.

MODE OF INFESTATION

Grasshoppers become infested, while feeding, by swallowing eggs of the parasite, and therefore they are vulnerable throughout their entire life. As a nymph grows older and its food consumption increases, its chance of becoming infested is correspondingly greater. The fact that a nymph becomes infested while young does not prevent it from acquiring additional parasites later in life. Grasshoppers are frequently found that harbor a hundred or more parasites of widely different ages.

When an egg reaches the alimentary tract the outer covering bearing the byssi has usually been rubbed off. The two opposite clear areas at the equator gradually become clearer and begin to protrude until they appear as colorless, hemispherical projections (fig. 1, *C*), which finally rupture and provide an opening for the escape of the larva (fig. 1, *D*). So far as has been determined, the larva in no way assists in its liberation. When first freed it is rather sluggish, although it quickly becomes active and soon penetrates the wall of the alimentary tract and enters the body cavity. This migration is discussed more fully in a later section.

The recently hatched larva (fig. 3) is about 740μ long by 34μ wide. A stylet of peculiar form is present, which can best be understood by reference to figure 1, *B*. The esophageal region, which comprises nearly half the length of the body, contains eight large esophageal glands (stichocytes) closely resembling those of *Agamermis decaudata*. The body ends posteriorly in a rounded terminus. A node and a postnodal region are lacking.

PARASITIC LAVAL STAGE

The parasitic development of the larva has not been carefully studied, but it seems essentially the same as that of *Agamermis*

decaudata. There is the same rapid increase in size and the same type of trophosome development.

Males usually remain in the host from 4 to 6 weeks and attain a length of from 20 to 60 mm. Females usually remain in the host from 8 to 10 weeks and attain a length of 50 to 160 mm. However, both the duration of the parasitic stage and the size of the fully grown larva are subject to great variation. In general the fewer parasites the insect harbors, the larger they become and the longer they remain in the host. Likewise there is a tendency for these parasites to grow larger and remain longer in a large grasshopper than in a small one.

The emergence of *Mermis subnigrescens* results in the death of the host. A grasshopper may harbor parasites of widely different ages, and when the oldest ones emerge most of the others perish with the host. However those that are almost fully grown may also emerge and enter the soil. The same thing happens when a parasitized grasshopper dies from some other cause: the parasites that are nearly full grown may emerge, the others will die. Many of these prematurely emerged individuals eventually die in the soil, but some undoubtedly survive. The parasites begin emerging at least by the first of July and continue throughout the summer as long as grasshoppers are alive. The emergence of *M. subnigrescens* from its host is similar to that described for *Agamermis decaudata*.[5]

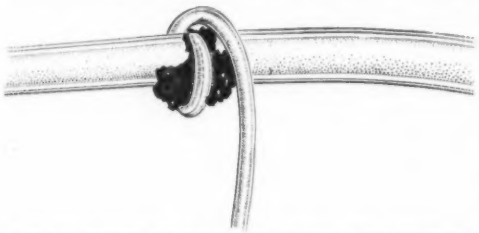

FIGURE 4.—Copulation in *Mermis subnigrescens*. (Freehand sketch.)

The final molt occurs the following spring. Experimental specimens buried at Woods Hole during the autumn of 1927 had molted or were in the process of molting when examined on April 12, 1928.

ADULT STAGE

Mermis subnigrescens is found in the soil at various depths down to about 24 inches. The majority occur from 6 to 18 inches below the surface. As a rule, individuals remain alone and one rarely finds a female and one or more males coiled into a knot as is characteristic of *Agamermis decaudata*. Copulation takes place at least occasionally and has been observed several times (fig. 4). By July females that emerged from their hosts the preceding summer begin to exhibit a brownish color owing to accumulating eggs, and by September they appear nearly black except for a short region at each extremity of the body. At this time the eggs are viable.

Oviposition takes place the following spring, beginning about the first of June and continuing until the first of August or possibly longer. In 1927 eggs were first found on the foliage May 27, but it was not until June 6 that they were numerous; in 1929 they were first found June 1, and by June 25 and thereafter they were numerous. Eggs are laid only during rains, and should there be no rain in June they will not be deposited in any number until rain occurs.

[5] CHRISTIE, J. R. See p. 187 of citation given in footnote 3.

Eggs are apparently not deposited during the night, a point that is discussed in a later section. At daybreak on a rainy morning in June or July ovipositing females are found moving about on the grass and other vegetation. If the rain continues, egg deposition goes on throughout the day, but if the rain ceases and the foliage becomes dry, the females coil up, fall to the surface of the ground, and enter the soil. Should the supply of eggs not be exhausted, a female presumably comes to the surface and resumes egg laying during a subsequent rain; however, the author was not able to demonstrate this experimentally. Before ovipositing, a gravid female 85 mm long contains approximately 14,000 eggs.

When laying eggs a female is entwined about a grass stem or other object (fig. 5), and the head end is extended farther and farther from

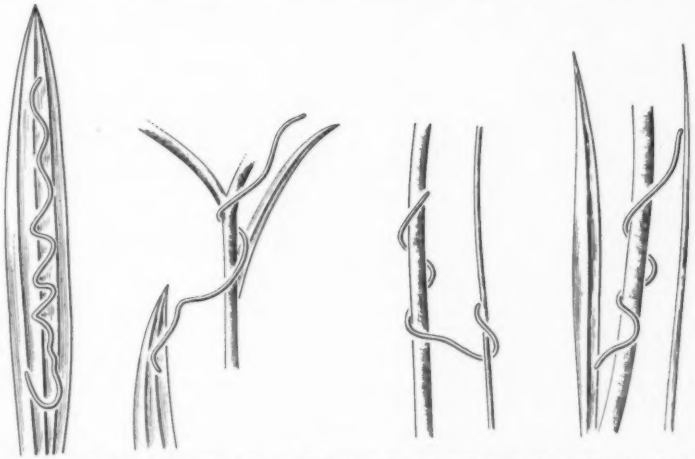

FIGURE 5.—Females of *Mermis subnigrescens* depositing eggs. (From freehand sketches made in the field.)

the supporting stem and is moved about with a more or less circular motion. If another stem is nearby, the anterior end is entwined at a point somewhat higher than the mermithid's position on the first stem. Thus, in dense vegetation a female, as it moves horizontally, is likely to mount higher and higher. However, if no object is nearby, more and more of the anterior end of the body extends until it begins to sag, and another or even the same stem may be entwined at a point nearer the ground.

How long a female lives after the uteri are emptied of eggs has not been determined. Since by this time the trophosome is nearly exhausted of reserve food materials, it seems unlikely that the female is able to survive another winter or to develop more eggs. However, if females are prevented from coming to the surface to deposit eggs, they will survive for several years.

Some of the males and females that emerged from hosts during September 1930, and were buried in containers at Woods Hole on September 25, 1930, were alive and in apparently good condition when examined on May 15, 1933. The females were filled with eggs, and there was no indication that any considerable number had been

deposited. Normally these individuals would have laid their eggs during June and July 1932, but being in containers they were unable to come to the surface. Sunlight is apparently the stimulus that causes egg laying. If an ovipositing female is placed in the dark, egg laying immediately stops; if again it is brought into the light, egg laying is promptly resumed. This interesting behavior has been described by Cobb.[6]

SECURING MATERIAL FOR STUDY

A limited number of postparasitic specimens of *Mermis subnigrescens* can usually be secured by digging, but this is a slow and laborious procedure. During the course of these investigations considerable digging was done not primarily for the purpose of securing material but to determine the abundance and distribution of the parasites in the soil. When securing material is the object, the operation can be facilitated by the use of sieves.

Adult females of *Mermis subnigrescens* can often be collected in large numbers while they are laying eggs. The collector should know the location of fields where these mermithids occur, and the fields must be visited during rainy days at the proper season of the year. On June 8, 1930, at Woods Hole, during a period of about 3 hours, the author collected 220 ovipositing females.

Probably the most practical method of obtaining *Mermis subnigrescens* in large numbers is to collect infested grasshoppers and confine them in cages until the parasites emerge. For this purpose any type of insect cage will serve if so constructed that 2 or 3 inches of soil can be placed in the bottom. In this soil the author usually planted wheat, which was allowed to grow a few inches high before the grasshoppers were placed in the cage. When one has less access to a field where there is a fairly high infestation a great many parasites can be secured in this manner. When grasshoppers are collected in fields and confined in cages a considerable number usually die. The death rate is highest during the first day or two and then gradually decreases. The result is many prematurely emerged parasites, which is the chief disadvantage of this method of securing material. It can be overcome to some extent by allowing the nematodes to remain in the soil of the cage for several weeks. During this time many of the prematurely emerged specimens die and are eliminated. When subsequently examined, additional abnormal specimens usually can be recognized and discarded. The soil in the cage must be kept moist at all times.

The specimens of *Mermis subnigrescens* thus obtained can be buried in the soil, where they proceed with their development and where they can be dug up and examined from time to time. One of the best containers for this purpose is an unglazed porcelain battery jar with the open end closed by 200-mesh, nickel alloy, wire cloth. Small jars for individual specimens can be obtained, or large ones that will accommodate 100 specimens or more. The soil used to fill the containers and in which the mermithids are placed should be composed in part of sand and should be passed through a sieve. When the container is subsequently dug up the mermithids may be secured by again passing the soil through a sieve. If the soil is

[6] COBB, N. A. THE CHROMATROPISM OF MERMIS SUBNIGRESCENS, A NEMIC PARASITE OF GRASSHOPPERS. Jour. Wash. Acad. Sci. 19: 159–166, illus. 1929.

partly sand and has previously been sieved this can be done with little danger of injury to the specimens.

For concentrating these parasites in a small area, outdoor enclosures were constructed, 4 to 6 feet wide by 8 to 12 feet long, with the sides and top covered with ordinary window screening. Grasshoppers collected in fields of high infestation were placed in these enclosures, which were so situated that a naturally luxuriant vegetation served as food for the insects. The mermithid population of the underlying soil was undoubtedly increased in this manner, but even here the securing of specimens by digging was slow and laborious.

If an adequate source of infested grasshoppers is not available, uninfested grasshoppers may be collected and artificially infested. Such a method could be employed should an attempt ever be made to colonize the parasite in regions where it does not exist. The following procedure has proved fairly satisfactory. Leaves of the buckhorn plantain (*Plantago lanceolata* L.) were secured, and on them eggs of the parasite were sprayed by means of a small atomizer. The stems of the plantain leaves were then inserted into a small-mouthed vessel containing water, in the same manner as flowers are placed in a vase, and transferred to an insect cage. Any convenient cage can be used, the size depending on the number of grasshoppers to be handled.

The chief difficulty with this and other methods employed for artificially infesting grasshoppers is to prevent too many eggs from being swallowed. So many mermithids per host are acquired that most of the parasites develop into males.[7] Grasshoppers infested by this method must be held in confinement and fed throughout the entire developmental period of their parasites, while in those already infested when collected many of the parasites may be almost ready to emerge.

TIME OF EGG DEPOSITION

Rain stimulates females of *Mermis subnigrescens* to ascend vegetation and deposit eggs, a fact which was clearly demonstrated at Woods Hole in 1929. The spring and early summer were exceptionally dry, and very little rain fell between June 1 and July 19. On a plot of land where *M. subnigrescens* was known to be abundant, only a few eggs had been found on the foliage. On July 17, a lawn sprinkler was operated from 4:30 p. m. until 8 p. m. During this period of 3½ hours an average of about 2 inches of water was applied over an area approximately 14 feet in diameter. Early the following morning 12 mermithids were seen depositing eggs on the vegetation in this area, and undoubtedly there were others that were overlooked. None were found outside the sprinkled area. On the following day the sprinkler was moved to a new position, and the operation was repeated with similar results. In both sprinkled areas a subsequent examination of the foliage showed that eggs were present in abundance.

It has already been mentioned that light is apparently the stimulus that causes egg laying, a fact which indicates that oviposition cannot take place at night. On July 19, 1929, four ovipositing females were kept under constant observation from 7:30 p. m. until 9:45 p. m.

[7] CHRISTIE, J. R. SOME OBSERVATIONS ON SEX IN THE MERMITHIDAE. Jour. Expt. Zool. 53: 59–76 illus. 1929.

During this time rain fell intermittently. When it became so dark as to make observation impossible a pocket flashlight was used occasionally. By 8:50 it was nearly dark, and by 9:20 it seemed totally dark. Throughout early twilight all four females continued their characteristic movements, but as darkness increased they became gradually less active. At 9:40, about 30 or 40 minutes after the last vestige of daylight had apparently disappeared, two of the females were still on the vegetation but had ceased moving about. The other two had fallen to the surface of the ground, where they remained partly coiled up and comparatively inactive. Not one appeared to make any attempt to enter the soil.

At intervals one of the females was removed from the vegetation and placed on a wet leaf, where it was allowed to remain for half a minute or a minute (table 1). The leaves were later examined and the eggs counted. The results appear to indicate further that eggs are not laid at night.

TABLE 1.—*Effect of darkness on rate of egg deposition by Mermis subnigrescens*

Time of day (p. m.) female was placed on leaf	Period left on leaf	Light conditions	Eggs found on leaf
			Number
7:45	½ minute	Early twilight	21
8:20	½ minute	Late twilight	18
8:50	½ minute	Almost dark	41
9:20	1 minute	Dark	41
9:40	1 minute	do	0

DISSEMINATION OF THE EGGS

Eggs of *Mermis subnigrescens* were found on any object over which the ovipositing female had passed, whether living foliage or dead branches. There was no evidence that females selected any particular kind of foliage on which to oviposit. Eggs were found on either side of a leaf, sometimes in masses of 25 or more and at other times widely scattered. They were largely confined to herbaceous vegetation to a height of about 2 feet. On a few occasions they were found on the leaves of shrubs about 3 feet from the ground. The author saw no indication of dissemination by wind.

To facilitate the examination of a large amount of vegetation for the presence of eggs, the following method was employed: A piece of corrugated glass about 3 inches wide by 8 inches long, with the corrugations running lengthwise, was held on an improvised stand, in an oblique position, high enough from the table to allow a beaker to be placed under the lower end. A second beaker, containing 20 to 30 cc of water, was at hand. A leaf from the sample of foliage being examined was held on the corrugated glass with the left hand. A camel's-hair brush, with the bristles cut off to about one-third their original length, was repeatedly dipped into the water and passed several times over both surfaces of the leaf. Thus the eggs were dislodged and carried by the water down the corrugations of the glass into the beaker below. When the sample of vegetation was finished the remaining water was poured over the corrugated glass to wash any adhering eggs into the beaker. The water was then examined for eggs, usually after having been reduced in bulk by centrifuging. This method served well to demonstrate the presence or absence of

eggs, and when eggs were present it indicated with fair accuracy their relative abundance.

During the summer of 1929 an intensive study was made of egg dissemination in the vicinity of Woods Hole and in some of the adjoining localities of southeastern Massachusetts. The data secured are not given in detail. Suffice it to say that in fields where the parasite was present eggs were very widely disseminated. Samples of vegetation taken from the roadside, from back yards, and from other apparently unpromising locations were found very frequently to carry a few eggs.

To determine the vertical distribution of eggs a sample of grass consisting of all that grew on 1 square foot was collected during July 1929. Each stalk was cut close to the ground, the butts were placed evenly together, and the whole was cut into 2-inch lengths. Each 2-inch length was treated as a separate sample; every piece of grass in it, whether leaf or stem, was washed, and the washings were examined for eggs. The results are shown in figure 6.

On July 12, 1929, collections of grass and plantain leaves were made in 11 fields between Pawtucket, R. I., and Franklin, Mass. Eggs were found in all except two collections. That one could go into a region where data were not available regarding the distribution of this nematode, take 11 samples of vegetation at random from widely separated fields, and find eggs on every sample except 2 is further evidence that the parasite is widely distributed.

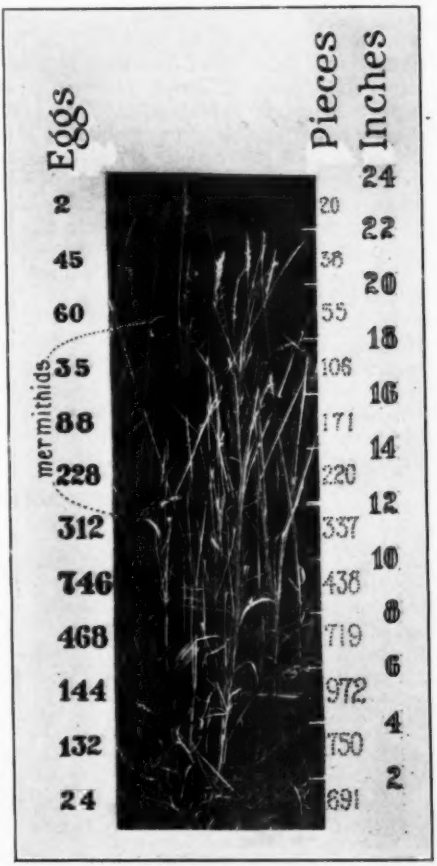

FIGURE 6.—Vertical distribution of eggs of *Mermis subnigrescens* on grass. The number of pieces of vegetation in each sample, the number of eggs found, and the height of each sample from the ground are indicated.

MIGRATION OF LARVAE FROM INTESTINE TO BODY CAVITY OF HOST

The hatching of *Mermis subnigrescens* eggs in the intestine of grasshoppers and the passage of the larvae through the intestinal wall into the body cavity were observed on several occasions. In making these

observations the following procedure was followed: Recently hatched nymphs of *Melanoplus femur-rubrum* (DeGeer) were placed in a cage and kept without food for 24 hours. A mash was prepared by mixing cracker crumbs, cane sugar, and orange juice. About 100 viable eggs were mixed with a bit of this mash to form a pellet a millimeter or so in diameter. This pellet and a grasshopper nymph were placed in a short glass tube corked at both ends. The nymph, when brought close to the pellet by patiently manipulating the tube, would usually eat. After a period of time varying from 30 minutes to 2 hours the nymph was killed, and its alimentary tract was removed and mounted on a slide under a cover glass in a drop of body-cavity fluid. A supply of adult grasshoppers was kept available to provide body-cavity fluid for this purpose. The preparation was then placed on the stage of a microscope for observation.

The first indication of hatching usually occurred after 1 to 1½ hours, when the eggs began to show the characteristic clear, hemispherical projections. Very soon after this the mermithid larvae were free in the intestine. At the end of 10 hours eggshells usually appeared in the feces. Most eggs hatched somewhere between the posterior end of the crop and the region where the Malpighian tubes are attached.

When first free from the shell the larva was comparatively inactive but soon began to move about. Sometimes 20 to 30 minutes elapsed before it passed into the body cavity. While penetrating the tissues of the intestinal wall the stylet was distinctly seen being rhythmically protruded. Most frequently larvae penetrated the intestine at the region immediately posterior to the caeca. On one occasion a larva entered a caecum and passed through its wall into the body cavity

EFFECT OF THE PARASITE ON THE HOST

The effect of *Agamermis decaudata* on the development of its host has been discussed in a previous paper.[8] In general the facts stated therein also apply to *Mermis subnigrescens*. The gonads of female grasshoppers are markedly inhibited in their development, and infested individuals are usually sterile. The effect on the development of the male gonads is less pronounced. In both sexes growth is materially retarded, infested individuals remaining in the nymphal stage longer than uninfested ones. A grasshopper is always killed when a parasite emerges. There is a tendency for the host to be older when it becomes infested than in the case of *A. decaudata*, and also for a host to harbor a greater number of parasites.

As already noted, *Mermis subnigrescens* apparently infests only grasshoppers. The following species were found naturally infested or were experimentally infested in the laboratory:

Species:	*Infestation*
Arphia sulphurea (Fabricius)	Experimental.
Camnula pellucida (Scudder)	Natural and experimental.
Chorthippus longicornis (Latreille)	Do.
Chortophaga viridifasciata (DeGeer)	Natural.
Conocephalus brevipennis (Scudder)	Do.
Encoptolophus sordidus (Burmeister)	Do.
Melanoplus bivittatus (Say)	Do.
M. femur-rubrum (DeGeer)	Natural and experimental.
M. mexicanus (Saussure)	Natural.
Orphulella pelidna (Burmeister)	Do.
Romalea microptera (Beauvois)	Experimental.

[8] CHRISTIE, J. R. See pp. 186–189 of citation given in footnote 3.

DISTRIBUTION IN NEW ENGLAND

Between August 18 and 21, 1930, Gerald Thorne and the author collected and examined grasshoppers along the Merrimack River Valley between Manchester, N. H., and Woodstock, N. H., along the Connecticut River Valley between Middletown, Conn., and Woodsville, N. H., and from several localities in the interlying country. Collections were made in 83 places, and the number of grasshoppers examined in each collection varied from 10 to 100, depending on their abundance. The results may be summarized as follows:

Collections of grasshoppers:	*Grasshoppers infested (percent)*
10	None.
30	1 to 9.
19	10 to 19.
11	20 to 29.
7	30 to 39.
3	40 to 49.
2	50 to 59.
1	80.

A total of 2,500 grasshoppers examined showed an average infestation of 12 percent. The grasshoppers were not identified, and the collections were made up of various species, with *Melanoplus femur-rubrum* usually predominating. The mermithids from 66 of the collections were identified by Thorne, to whom the author is indebted for this information. In 42 collections the parasites were exclusively *Mermis subnigrescens;* in 17 collections both *M. subnigrescens* and *Agamermis decaudata* were present; in 6 collections the parasites were all *A. decaudata;* and in one collection *M. subnigrescens, A. decaudata,* and a species of *Hexamermis* were present.

About the same conditions exist in Barnstable, Bristol, and Plymouth Counties, Mass., where the author has made many collections of grasshoppers. *Mermis subnigrescens* is the most common mermithid parasite of grasshoppers in New England, and in this region it is very widespread. There are few fields where its presence cannot be demonstrated.

ECONOMIC SIGNIFICANCE

There is little doubt that both *Mermis subnigrescens* and *Agamermis decaudata* are important factors in grasshopper control throughout the regions where these parasites occur. Of the two, *M. subnigrescens* is perhaps the more important. It appears to be able to withstand a greater variety of soil and climatic conditions and to maintain itself in larger numbers where the grasshopper population is consistently low. It does not extend as far south as does *A. decaudata.* Insufficient rainfall has undoubtedly prevented the spread of both species into semiarid regions. Throughout much of the area in the United States where they are not found their introduction is evidently out of the question. Nevertheless there may be isolated regions where they do not exist but where climatic conditions are such that they could be established. Of the two species, *M. subnigrescens* is probably the best suited for colonizing. Its eggs are easily secured in large numbers, remain viable for months, and can be shipped any distance. The artificial infestation of indigenous grasshoppers should be a comparatively simple matter.

SUMMARY

Mermis subnigrescens Cobb, a common parasite of grasshoppers, occurs throughout the northeastern part of the United States and westward as far as Minnesota, Iowa, and Missouri. The present paper is based on studies made at Woods Hole, Mass.

The adults of this parasite occur in the soil. On rainy days during June and July gravid females come to the surface of the ground, ascend low herbaceous vegetation, and deposit their eggs. The eggs adhere to foliage by means of the entangling appendages or byssi. Grasshoppers, while feeding, swallow the eggs, which hatch in the alimentary tract. The larvae immediately migrate through the wall of the alimentary tract into the body cavity. They remain in the host from 4 to 10 weeks to complete their growth; then they emerge by forcing their way through the body wall and enter the soil. They molt the next spring, pass the ensuing summer and winter in the soil, and the females deposit their eggs during the following summer.

THE PRODUCTION OF CITRUS MOTTLE-LEAF IN CONTROLLED NUTRIENT CULTURES [1]

By H. D. CHAPMAN and A. P. VANSELOW, *assistant chemists*, and GEORGE F. LIEBIG, JR., *associate in the experiment station, California Agricultural Experiment Station*

INTRODUCTION

Although it is now definitely established that mottle-leaf of citrus can be controlled by the use of zinc sprays (*5*, *7*),[2] little is known about the true nature of this disease, the physiological function of zinc, and kindred questions. One approach to the solution of these problems lies in determining the nutrient and environmental conditions under which this disturbance can be developed artificially. Except for some indications recently reported by Haas (*3*) previous attempts to produce mottle-leaf of citrus under the known conditions of solution or sand culture have been unsuccessful. Hoagland, Chandler, and Hibbard (*4*) have produced little leaf of apricot trees and zinc-deficiency symtoms in other plants by the omission of the zinc from culture solution, when special efforts were made to minimize zinc contamination. However, trials with citrus gave negative results under similar conditions.[3] This, they thought, might in part be due to the low light intensities prevailing at Berkeley, Calif.

Accordingly, a series of experiments with citrus were set up at Riverside, Calif., in March 1936. The positive results of these trials, together with several interesting observations, appear to justify a progress report at this time.

DESCRIPTION OF EXPERIMENTS

The technique of these experiments is given in some detail for the benefit of those engaged in similar or related studies.

Leafy twig cuttings from healthy, vigorous, Valencia orange trees were rooted in coarse-grained sand and then transferred to pyrex-glass battery jars containing nutrient solutions of the desired composition. The cuttings were held in place by specially painted wooden plugs inserted in appropriately spaced holes in Bakelite covers. The sugar pine plugs were first painted with a high-grade asphaltum varnish and then with two coats of a synthetic Bakelite-type varnish. Previous work has shown that both Bakelite and the varnishes used are highly resistant to the deteriorating action of weak salt solutions and at the same time yield no toxic products to the culture solution. Although the ash of these materials contained traces of zinc and various other inorganic constituents (table 1), the resinous matrix of

[1] Received for publication Feb. 23, 1937; issued September 1937. Paper no. 366, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 378.

[3] Unpublished observations communicated to the authors by D. R. Hoagland.

TABLE 1.—*Impurities in major chemicals (c. p. grade) and accessory materials*

Material	Manufacturer or agent	Lot no.	Impurities as determined spectrographically		
			Zinc content	Definite traces	Just detectable
			P. p. m.		
K_2SO_4	General Chemical Co.	5	3.5		Bi, Ca, Cu, Mg, Pb, Sn, Si.
KNO_3	J. T. Baker	41834	1.0	Bi	Ca, Cu, Pb, Sn, Si.
$Ca(NO_3)_2.4H_2O$	Merck	30553	1.0	Ag, Ca, Fe, Pb, Sn	Bi, B, Cu, Mg, Si.
$CaSO_4.2H_2O$	do	31791	1.0	Cu, Pb	Ba, Bi, Mg, Ni, Si, Sn.
K_2PO_4	J. T. Baker	11623	3.5	As, Bi, Ca, Cu, Mg, Pb, Sb, Si, Sn.	Ag, Hg.
$MgSO_4.7H_2O$	Merck	31433	2.0	Ag, Cu, Mg	Bi, Pb, Sn.
$Mg(NO_3)_2.6H_2O$	J. T. Baker	41934	8.0	Cu, Mg, Pb, Sn	Bi, Si.
$FeSO_4$	do	72426	20.0	Cu, Bi, Mg, Pb, Si, Sn	Ti.
KOH	do	121521		Ca, Na	Sr.
$KHSO_4$	do	51530		Ca	
NaOH	Mallinckrodt	1177126		Al, Ca	Fe, K, Mg, Sr.
Bakelite	Bakelite Corporation.			Al, Ca, Cu, Mg, Zn.	
Asphalt paint (Gila coat).	Fuller & Co			Ca, Pb, Ag, Zn.	
Phenolic resin varnish.	Du Pont			Pb, Ca, Co, Cu, Mg, Mn.	Al, Fe, Ni, Si.

the paint prevents appreciable solution of these elements. Before being used the battery jars, Bakelite covers, and painted plugs were digested with 20-percent hydrochloric acid. In order to avoid contamination of the nutrient solution with dust from the air, the covers were held tightly to the ground edge of the battery jars by springs attached to a metal harness surrounding the jars. The battery jars were coated on the outside with an asphaltum, followed by an aluminum varnish. During the summer it became necessary to reduce the solution temperatures within the jars by insulation with sphagnum moss. A portion of the general set-up is shown in figure 1.

All water used in the experiment was redistilled in an all-pyrex-glass still designed for continuous operation.

Zinc was quantitatively determined in all of the major chemicals used by the method of Vanselow and Laurance (*11*). With the exception of the iron sulphate, they were found to contain so little zinc that purification was not attempted (table 1).

Because of the precautions taken to prevent contamination and the fact that plant stimulation has often been produced by many other minor elements, 54 trace elements were added as a basic treatment to most of the cultures. A moderately concentrated stock solution of each was made up separately, and from these, secondary mixtures of compatible combinations.

The kinds and amounts of these minor elements added to the final culture solution, the source from which derived, and the results of a qualitative spectrographic determination [4] of the impurities contained by these chemicals are given in table 2.

The impurities carried by the major salts and several accessory compounds are shown in table 1. All acids used in the experiments were redistilled in a pyrex-glass still.

[4] These determinations were made by means of a concave grating spectrograph (*11*), samples of the dry material being arced on graphite rods.

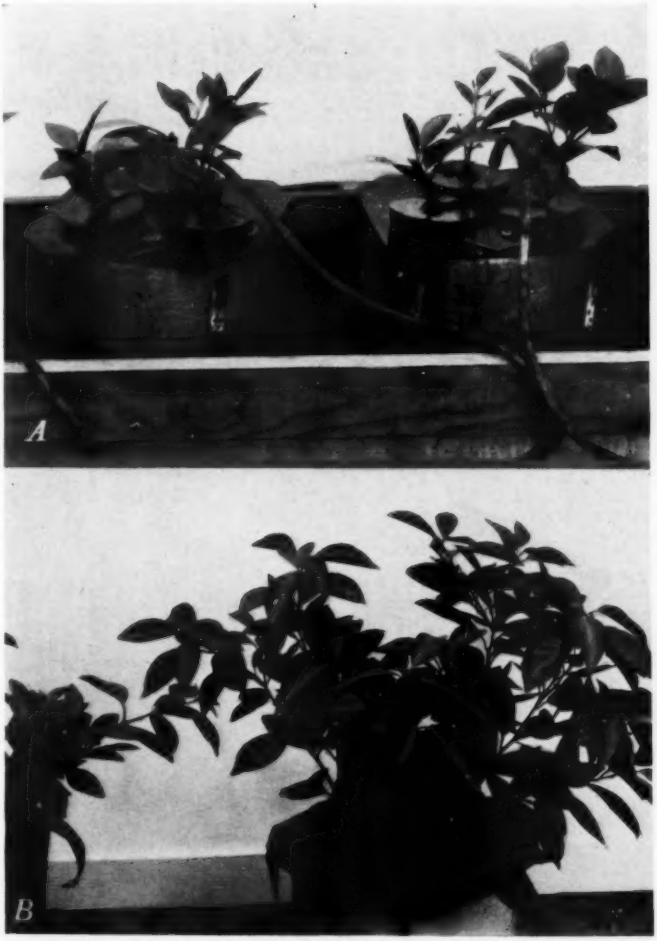

FIGURE 1.—Typical cuttings in the experiment showing their size after emergence of first growth cycle (*A*) and again after third growth cycle (*B*).

TABLE 2.—*Trace elements added to culture solution, and impurities in chemicals from which derived*

Element	Amount added to final culture solution		Source	Manufacturer or agent	Grade	Lot no.	Impurities as shown spectrographically	
	Per liter	γ A.[1]					Definite traces	Just detectable
	Milligrams							
Al	0.539	20.0	$KAl(SO_4)_2.12H_2O$	J. T. Baker	C. p.	10722		Ca.
Sb	.012	.1	Sb_2O_3	do	do	11629		Al, Ca, Mg, Pb, Si.
As	.007	.1	As_2O_3	do	do	8226	Sb	Ca, Si.
Ba	.686	5.0	$BaCl_2.2H_2O$	do	do	12231	Ca, Sr	Cu, Fe, Mg, Ni, Pb, Si.
Be	.090	10.0	$Be(NO_3)_2.3H_2O$	Eimer & Amend	do	H100	Ca, Mg, Si	Ba, Na, Ti.
Bi	.021	.1	Metal	Coleman & Bell	do			Ca, Pb, Si.
B	.541	50.0	H_3BO_3	Merck	do	30343		Si.
Br	1.598	20.0	NaBr	J. T. Baker	do	61522	Ca	Al, Ba, Sr, Ti, V.
Cd	.011	.1	Metal					Ca, Cu, Mg, Pb, Si.
Ce	.014	.1	$CeCl_3$	Kahlbaum		X2CI	Ca, Mg, Na, Sr	Cu, Si, Sn, Th, V.
Cs	.013	.1	CsCl	do		Z1309	Na, Rb	Ca, K, Mg, Sr.
Cl	7.092	200.0	NaCl	J. T. Baker	C. p.	8530	Mg	Ba, Ca.
Cr	.052	1.0	$K_2Cr_2O_7$	Merck	do	30793	Ca	Co, Pb, Sr.
Co	.005	.1	$CoCl_2.6H_2O$	J. T. Baker	do	31227	Ca, Mn, Ti, Ni	Cu, Fe, K, Mg, V.
Cb	.009	.1	Cb_2O_5	A. P. Vanselow			Pt, Si, Ti	Ca, Cr, Cu, Mg, Sn, Ta, W, Zr.
Cu	.063	1.0	$CuSO_4.5H_2O$	Merck	C. p.	30663	Ni	B, Ca, Fe, Mg, Pb, Si.
Er	.050	.1	$Er(NO_3)_3xH_2O$	Eimer & Amend	do	A27	Al, Ho, Lu, Mg, Pb, Zn, Ca, Dy, Tm , Yt, Yb	Ba, Ce, Cu, Nd, Sm, Si, Sn.
F	.019	1.0	NaF	J. T. Baker	do	81622	Ca, Sr	Al, Ba, Cu, Mg, Pb, Sn, Ti.
Ga	.006	.1	Metal	A. D. Mackay			Cu, Mg, Ti, Tl, V, Zn	Al, Ba, Ca, Si, Sn, Sr.
Ge	.007	.1	GeO_2	Eimer & Amend		B36	Al, Ba, Ca, Mg, Na, Si	Cu, Fe, Sr, Ti.
Au	.019	.1	$AuCl_3$	University of California			Ca, Si	Cu, Mg.
In	.011	.1	Metal	A. D. Mackay			Cu, Pb, Zn	Ag, Al, Ba, Bi, Cu, Ga, Mg, Si, Sr.
I	.012	.1	NaI	Merck	Reagent	30254		Ca, Mg.
Ir	.019	.1	$IrCl_3$	Eimer & Amend		D37	Fe, Ru, Sr, Ti, Ca	Ag, Al, Ba, Co, Cu, Mg, Ni, Pt, Si.
La	.013	.1	$LaCl_3$	do	C. p.	A27	Ca, Fe	Cu, Pb.
Pb	.020	.1	$2PbCO_3.Pb(OH)_2$	J. T. Baker	do	1019	Ca	Al, Mg, Si.
Li	.069	10.0	$LiCO_3$	do	do	111322	Ca, Sr	Al, Fe, Na, Mg, Ti.
Mn	.549	10.0	$MnCl_2.4H_2O$	Eimer & Amend	Tested purity	H94	Ca	Al, Ba, Cu, Fe, Mg, Ni, Pb, Si, Sn, Sr.
Hg	.020	.1	$HgCl_2$	Merck	C. p.	30692		Ca.
Mo	.009	.1	MoO_3	J. T. Baker	do	11429	Ca	W.
Nd	.014	.1	$NdCl_3$	Eimer & Amend	do	C47	Ca, Dy, Pr, Sa, Tm	Ag, Al, Ce, Cu, Er, Gd, La, Mg, Sc, Si, Ta, Zr.
Ni	.005	.1	$NiCl_2.6H_2O$	do	Tested purity	B38	Ca, Mn, Dy, Ti, Co	Cu, Fe, Mg, Si.
Os	.019	.1	OsO_4	do				

Pd	.610	.1	$PdCl_2.2H_2O$	A. H. Thomas Co.			Ca, Cu, Au	Ag, Fe, Rh, Mg, Si, Ru.
Pt	.019	.1	K_2PtCl_6	Eimer & Amend	C. p	E21	Ca	Ag, Al, Au, Si, Ti, V.
Rh	.010	.1	$RhCl_3$	?	?	?	Ag, Ca	Cu, Si, Ti, Zr, Mg.
Ru	.010	.1	$RuCl_3$	?	?	?	Ca, Ti, Zn, Rn?	Ag, Al, Cu, Fe, Rh, Mg, Si, V, Ta.
Rb	.008	.1	Rb_2SO_4	Eimer & Amend	C. p	D45	K	Ca, Mg, Na.
Se	.079	1.0	Na_2SeO_3	E. H. Sargent	do		Ca	Al, Mg, Pb.
Si	1.400	50.0	SiO_2	J. T. Baker	do	72527	Al, Fe, Mg, Na, Ti	Ba, Ca, Cu, Sr.
Ag	.010	.1	Ag_2SO_4	do	do	32927		Ca, Mg, Si.
Na	4.600	200.0	NaCl	do	do	8530	Mg	Ba, Ca.
Sr	.087	1.0	$SrCO_3$	Cenco	do	1223	Ca, Ba, Mg	Cu, Mn, Na, Si.
Ta	.018	.1	Metal	A. D. Mackay			Ca, Si	Al, B, Ni, Ti.
Te	.012	.1	Na_2TeO_3	Eimer & Amend	Pure	C97	Ag, Al, Ca, Cu, Pb	Ba, Fe, K, Mg.
Tl	.020	.1	$TlNO_3$	Cenco	C. p		Fe, Mg	Al, Ca, Cu, Mn, Pb.
Th	.023	.1	$ThCl_4$	Will Corporation	do		Zr	Ca, Ce, Cu, Mg, Si, Ti.
Sn	.011	.1	$SnCl_2.2H_2O$	J. T. Baker	do	22230	Pb	Al, Ca, Cu, Fe, In, Mg, Si.
Ti	.239	5.0	TiO_2	Eimer & Amend	do	B38		Ca, Cu, Mg, Pb, Si.
W	.018	.1	WO_3	J. T. Baker		4126		Ba, Ca, Cr, Cu.
U	.023	.1	$UO_3.(C_2H_3O_2)_2.2H_2O$	do	C. p	102333	Mg, Th	Ba, Ca, Na, Sn, Zn.
V	.059	1.0	NH_4VO_3	Eimer & Amend	do	B36		B, Ba, Ca, Si.
Yt	.008	.1	$Yt(NO_3)_3.6H_2O$	do		D37	Ba, Dy, Er, Sm, Zr, Ca, Yb.	Al, Ce, Gd, Pb, Cu, Fe, Mg, Nd, Si, V.
Zr	.009	.1	$Zr(NO_3)_4.5H_2O$	do	C. p	D123	Ca, Si, Ti	Mg, Pb.

[1] One millionth of a gram-atom.

The field observation that both nutrient and light conditions apparently modify the zinc requirement of citrus suggested that the cultures with and without zinc be duplicated under such conditions as to render both of the above factors variable. The treatments as finally set up are given in table 3. The cultures designated as "low light"

TABLE 3.—*Nature of treatments under different light intensities*

Light intensity	Nature of culture solution		Culture no.
	Major elements	Minor elements	
Low (about 60 percent less than out of doors [1]).	Low NO_3	All,[2] no zinc	1 and 2.
		All, plus zinc (0.65 p. p. m.)	5 and 6.
	High NO_3	All, no zinc	9 and 10.
		All, plus zinc (0.65 p. p. m.)	13 and 14.
High (about 20 percent less than out of doors).	Low NO_3	All, no zinc	3 and 4.
		All, plus zinc (0.65 p. p. m.)	7 and 8.
	High NO_3	All, no zinc	11 and 12.
		do	21 and 22.
		All, plus zinc (0.65 p. p. m.)	15 and 16.
		None except Mn, B, Na, Cl, no zinc.	17 and 18.
		Double strength, all, no zinc	19 and 20.
	High NO_3 (purified by Steinberg method).	All, no zinc	23 and 24.

[1] Measured with Weston photographic exposure meter.
[2] Designates 54 trace element combination given in table 2.

were placed under a small lath shelter within the greenhouse, where the light intensity was reduced to about one-half that prevailing in other parts of the greenhouse. To secure information on the combined effects of the trace elements, cultures with no minor elements present except iron, manganese, boron, sodium, and chlorine, and with double the amounts of all but iron were included. Also, two cultures were purified according to the Steinberg (*10*) method by boiling with an excess of calcium carbonate. (Only the major salts were present at the time of purification, the trace elements being added to the purified solution.)

The composition of the culture solution as regards the major elements is shown in table 4. Iron sulphate, from which the zinc had

TABLE 4.—*Major components of low- and high-nitrate culture solutions*

Type of solution	Salt used	Quantity per liter	Quantity of indicated elements or radicals per liter					
			Ca	Mg	K	NO_3	SO_4	PO_4
		Grams	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*
Low nitrate	K_3PO_4	0.0212			0.30			0.10
	$Ca(NO_3)_2.4H_2O$	.2395	1.00			2.0		
	$CaSO_4.2H_2O$	.1331					0.77	
	$MgSO_4.7H_2O$	.1750	.77	0.71			.71	
	K_2SO_4	.1768			2.03		1.01	
	Total		1.77	.71	2.33	2.0	2.49	.10
High nitrate	K_3PO_4	.0212			.30			.10
	$Ca(NO_3)_2.4H_2O$	1.4848	6.27			12.55		
	KNO_3	.4974			4.92	4.92		
	$Mg(NO_3)_2.6H_2O$	.3244		1.26		2.53		
	$MgSO_4.7H_2O$	.6162		2.50			2.50	
	Total		6.27	3.76	5.22	20.00	2.50	.10

been removed by precipitation with hydrogen sulphide, according to the Vanselow and Laurance (*11*) method, was added daily at the rate of 0.2 mg iron per liter.

Previous experiments by Haas (*2*) with Valencia orange cuttings have indicated that an intermittent rather than a continuous supply of phosphate is conducive to better growth.

Accordingly, throughout the early stages of this experiment, phosphate was alternately added and withheld biweekly. The cultures were aerated continuously, the compressed air being introduced through sintered glass disks fused into the ends of pyrex-glass tubes.

By the addition either of nitric acid or potassium hydroxide, the pH of the solutions was kept between 6.5 and 7.0.

The culture solutions were at first renewed every month, and later every 3 weeks. Water lost by transpiration and evaporation was replaced daily by the addition of redistilled water. After the experiment had progressed for some time, quantitative determinations of zinc were made in both the fresh and the used solutions. The results of these determinations are as follows:

	Zinc per liter, milligrams
Fresh solution	0.007
Used solution (1 month old)	.003

RESULTS

The cuttings were transplanted from the propagating bed into the culture solutions on March 18, 1936, after the roots had been thoroughly rinsed in distilled water. In less than 12 days new roots began to develop and shortly thereafter new shoots appeared. By April 28 the leaves and shoots of the first growth cycle had attained full size, though the leaves were as yet somewhat tender and immature. None of the new leaves showed signs of mottle-leaf at this stage. The low-light plants appeared somewhat more healthy than the high-light plants and those grown in high nitrate were more vigorous than those grown in low nitrate.

By June 17 a second cycle of growth had emerged, the leaves and shoots having reached about the same stage of maturity as that of the first cycle on April 28. In contrast to the appearance of first growth cycle at this stage, the leaves of the plants in the cultures without zinc were more yellowish, some of them suggesting nitrogen deficiency; others showed some venation as in iron deficiency. Characteristic, though faint, mottle-leaf markings had developed in many of the now mature first-cycle leaves of the plants growing in minus-zinc, high-light cultures. There was no mottle, however, in any of the plants of the low-light series nor were any of the plants of cultures receiving zinc affected. By the middle of July the second growth cycle was virtually mature, and the leaves, which on June 17 had appeared yellowish, had become strikingly mottled. The plants of cultures 9 and 10 (low-light, high-nitrate, no-zinc) were only slightly mottled as compared with the more severe condition of corresponding cultures subjected to the full light of the greenhouse.

After the first 2 months of this experiment the roots of some of the plants in various cultures began to rot. This type of rot (gelatinization) always began on the very youngest roots, gradually spreading to older roots. At no time during this experiment was it possible to

correlate positively the incidence of this root rot with zinc, nitrate, light, or trace-element variables; on the other hand, it became increasingly evident as the experiment progressed that there was some relation between the phosphate status of the nutrient medium and the root rot. When phosphate was present the rotting usually became intensified, whereas when phosphate was omitted it tended to remain unchanged. In an effort to control the root rotting it was decided in June to decrease the concentration of phosphate to one-half of that employed at the outset.

Toward the latter end of July a third growth cycle began to emerge on all those plants whose roots were still in a healthy condition. In contrast to the early chlorotic appearance of the second growth cycle, this new cycle was green and vigorous. Moreover, the mottled leaves of affected plants began to turn green. A possible phosphate-zinc relation being suspected, phosphate was restored to its original level and kept present continuously until the termination of the experiment on September 15. As a result of this latter change, the leaves of the new third cycle soon began to acquire a yellowish cast, the venation of some of them becoming marked. By the middle of September the third cycle growth was virtually mature, the leaves of the plants in the minus-zinc cultures having become typically mottled.

Inasmuch as the roots of all save those in one of the plus-zinc, high-light cultures began to rot at the time the second change in phosphate technique was inaugurated, it was decided to make frequent small additions of zinc to one culture (no. 24). In contrast to the behavior of all the plants grown in the solutions lacking zinc, the third growth cycle of the plants in this culture remained healthy, the leaves attaining full size and becoming fully green. Moreover, the leaves of the second cycle, which on July 15 were badly mottled, became fully green. Representative leaves from the first, second, and third growth cycles of a plant from culture no. 24 are shown in figure 2. The first growth-cycle leaves (*C*) were at no time more than faintly mottled. The second-cycle leaves, *B*, formerly severely mottled, became completely green though there was no increase in leaf size.[5] The leaves *A* from the third cycle of growth were fully green and normal sized.

Owing to the increasing incidence of root rot in cultures hitherto healthy (occasioned, no doubt, by the continued presence of phosphate) the experiment was terminated on September 15.

An idea as to the growth made by healthy cuttings during the 6 months of this experiment may be had by reference to figure 1, *B*. Pictures of a few leaves from minus-zinc cultures showing some of the variations on leaf markings and sizes are presented in figure 3. The top row (*A*) shows mildly affected leaves taken from plants grown under low light conditions. The leaves in figure 3, *B*, show the more pronounced mottling developed under high light. That these leaves represent typical mottle-leaf may be seen by comparison with those shown in figures 4 and 5.[6]

The production of mottle-leaf under controlled conditions has made it possible to observe in detail the various stages in the development

[5] The greening of mottled leaves following zinc addition to the culture medium accords with the observation of Parker (8) and Reed and Parker (9) on the effects of zinc sulphate sprays.

[6] At the authors' request these latter were picked from orchard trees by E. R. Parker. At the time they were selected, the corresponding foliage of adjacent zinc-sulphate-treated trees was virtually free from leaves with markings such as those depicted in figs. 4 and 5.

of what is commonly regarded as the typical mottle-leaf pattern. The immature stage of leaves destined to become mottled is often characterized by a pronounced venation, the general color of the leaf being yellowish (fig. 6, two top leaves). As the leaf matures, the green areas adjacent to the veins spread and deepen in color and the

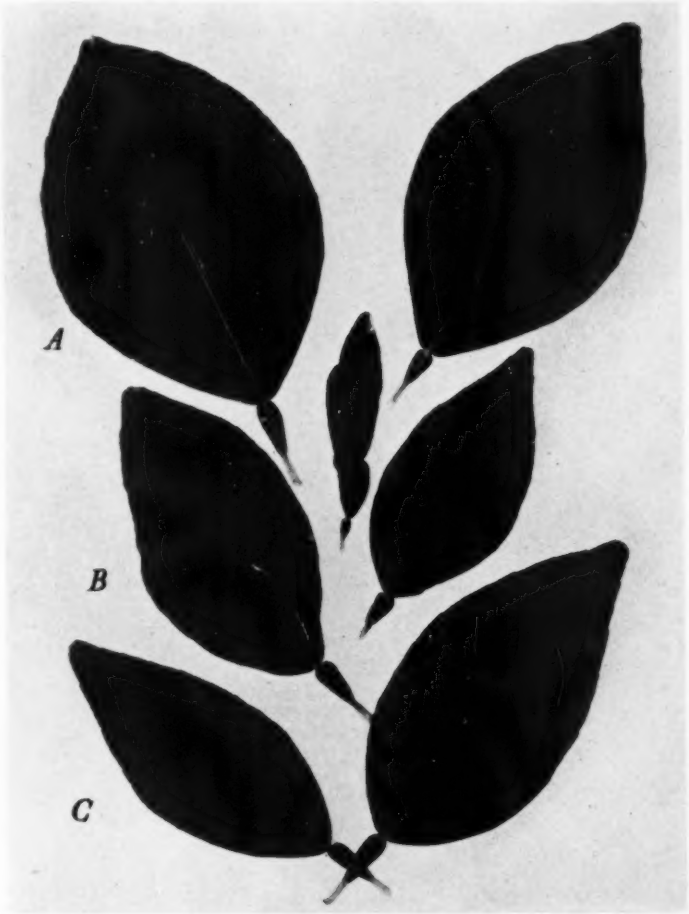

FIGURE 2.—Leaves from plant of culture no. 24: *A*, Third-cycle leaves which emerged green and healthy following zinc additions; *B*, second-cycle leaves formerly severely mottled, but caused to turn green following the addition of zinc; *C*, first-cycle leaves.

yellow-green areas between the veins become more pronouncedly yellow, thus giving rise to strikingly "mottled" patterns, often quite variable but with an element of similarity throughout. Small green spots are often seen in the yellowed areas. In more severe stages, the immature leaves are often uniformly yellow, the lower portion

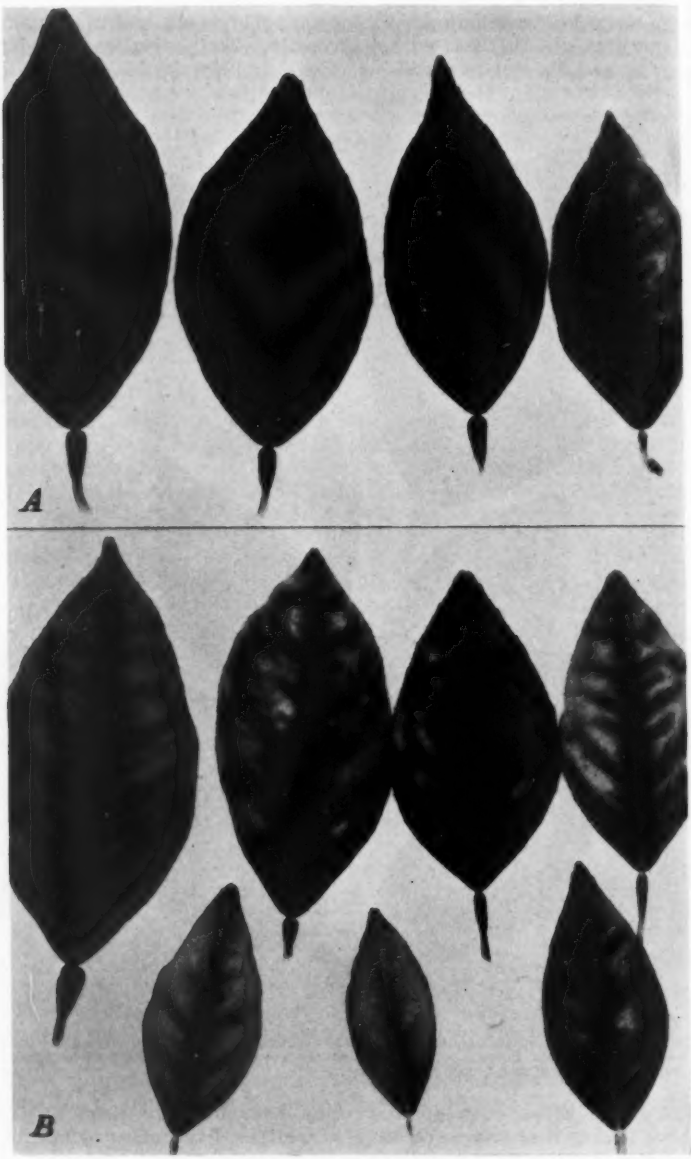

FIGURE 3.—Representative mottled leaves produced in minus-zinc cultures: *A*, Mild mottling produced under low-light conditions; *B*, the more severe mottling produced under higher light intensities.

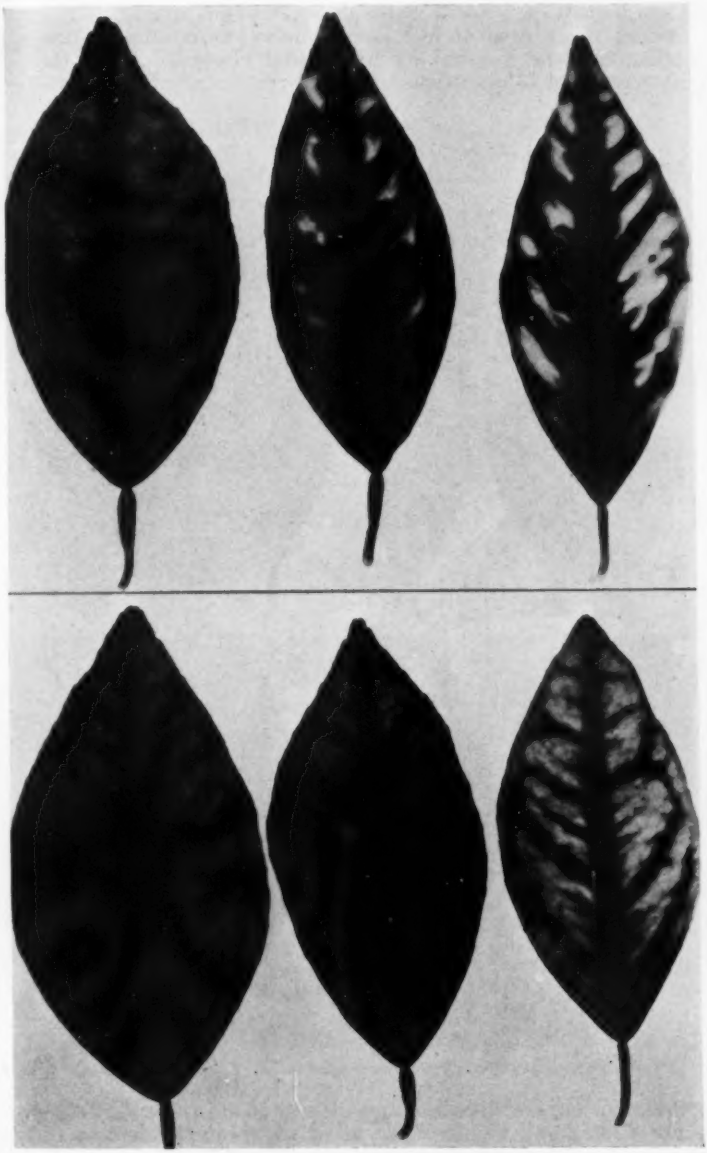

FIGURE 4.—Leaves from mottle-leaf-affected trees, showing some of the variations in size and markings.

of the veinal system being the only green part of the mature leaf. In mild cases the new growth may emerge entirely green, and only as the leaf matures will small yellow-green blotches appear.

During the course of leaf development, variations in degree of mottling may be induced by nutritional changes. Thus the continuous presence of phosphate under the conditions of this experiment

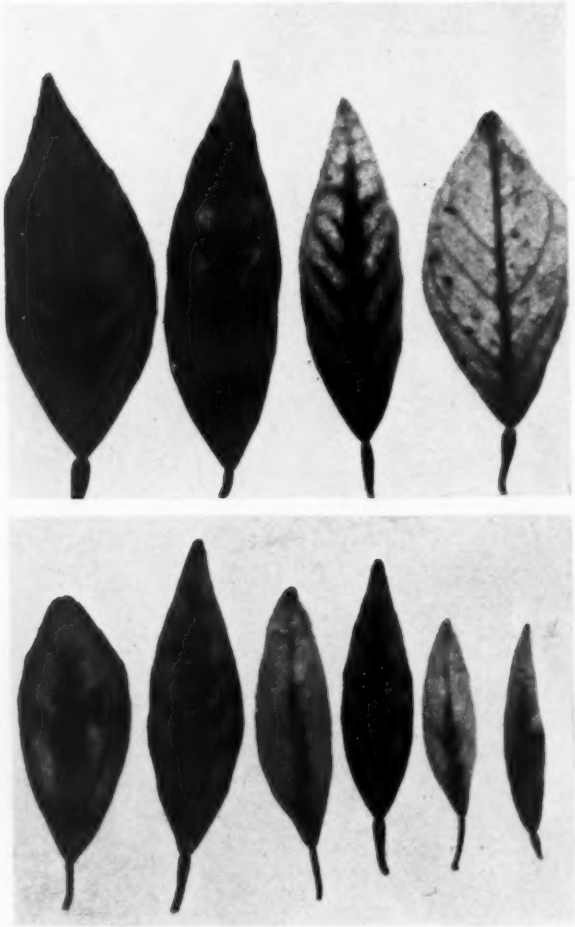

FIGURE 5.—Leaves from mottle-leaf-affected trees showing some of the variations in size and markings

caused immature but full-sized green leaves to mottle as they matured. On the other hand the frequent addition of small amounts of zinc caused mottled leaves to become green. However, at no time during the course of these experiments did any of the fully mature green leaves of the original cuttings become mottled, despite the development of

pronounced mottle-leaf in subsequent new growth. Cases of a similar nature on mature trees growing in orchards are often seen. This suggests that the zinc of mature leaves is rather immobile, as in the case of iron.

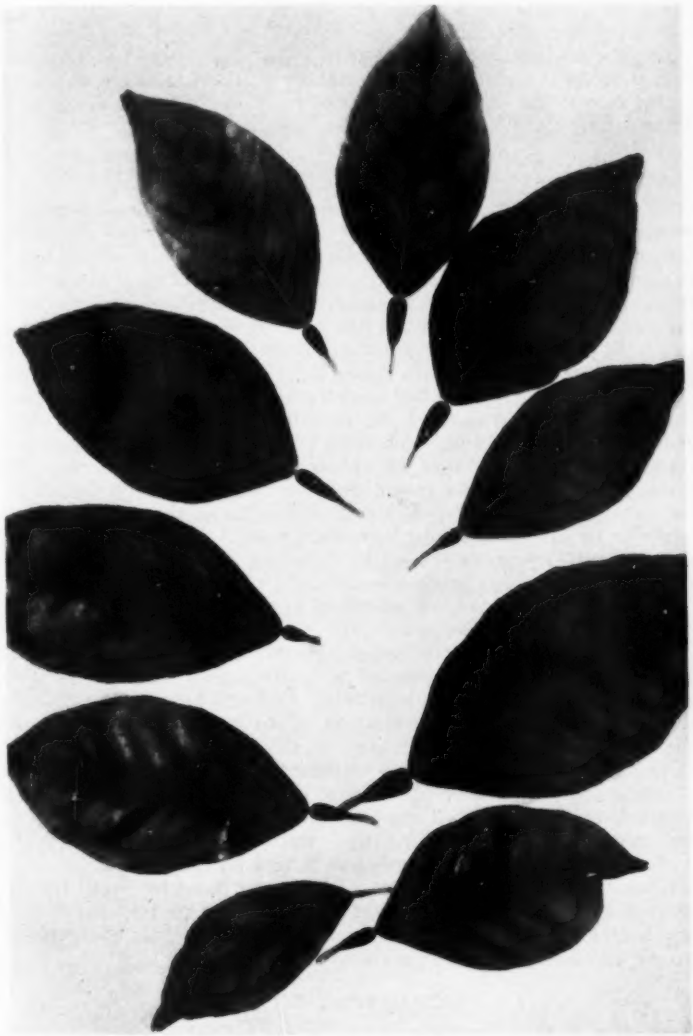

FIGURE 6.—Progressive stages in the development of typical mottle-leaf. Two top leaves show immature stage, the others progressive stages as the leaf matures.

It is of interest to note the effects of the trace elements. Where used in double strength, as indicated in table 3 (cultures nos. 19 and

20), top and root growth was definitely depressed though the leaves showed no localized evidence of injury. The roots were somewhat brownish and stubby. The plants of those cultures receiving zinc in addition to the 54 trace elements were healthy in appearance and showed no evidence of injury. It is unfortunate that, for purposes of comparison, cultures with zinc but minus the trace elements were not included in this experiment.

Despite the high boron content of pyrex glass plus the addition of 0.5 p. p. m. of this element to the culture solution, definite, though not severe, symptoms of boron deficiency (*1*) appeared in some of the plants during the course of the experiment.

CONCLUSIONS

Under the conditions of this experiment it has been possible to produce mottle-leaf of citrus by omitting zinc from the culture solution. Conversely, the addition of zinc not only prevented the development of mottle-leaf, but small amounts of zinc supplied frequently to plants already affected with mottle-leaf brought about recovery. Plants grown in the full light of the greenhouse (intensity about 80 percent of that out-of-doors) became severely mottled, whereas those subjected to a lower light intensity (about 40 percent of that out-of-doors) were but slightly affected. These results are in accord with the field observation that the leaves on the south side of citrus trees are usually more mottled than those on the north side.

During the early stages of this experiment there were definite evidences that the plants grown under conditions of high nitrate were more mottled than those grown with low nitrate, though in the latter there was no evidence of nitrogen starvation. However, the gradual decrease in the numbers of healthy cultures, owing to the incidence of root rot, has left this question unsettled.

Apparently the degree of mottling can be strongly influenced by the phosphate content of the nutrient medium. Increases in phosphate accentuate the mottling. Whether this effect is due to decreased zinc solubility in the culture solution, or to decreased availability within the plant, cannot be stated definitely. Olsen (*6*) has shown that high phosphate will induce iron chlorosis in certain plants through reactions within the plants. Zinc may be similarly affected.

Although mottle-leaf and root rotting were both accentuated by the continued presence of phosphate, there seems to be no necessary relation between these two conditions. On the one hand, mottle-leaf developed in plants that at no time were affected with root rot; on the other, root rot occurred unaccompanied by mottle-leaf.

The results of this experiment are more in harmony with the view that zinc is an indispensable plant food element, mottle-leaf of citrus being a manifestation of this deficiency, than that this element functions as an antiseptic or corrective.

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NUTRITIVE VALUE FOR DAIRY COWS OF ALFALFA HAY INJURED BY SULPHUR DIOXIDE [1]

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INTRODUCTION

In the vicinity of some industrial plants, the concentration of sulphur dioxide in the atmosphere becomes sufficiently high to injure the vegetation. Alfalfa (*Medicago sativa* L.) is one of the plants that is most susceptible to injury from this gas.

The injury to plants is of two types, chlorotic and acute (*5*).[3] The chlorotic type, which appears several days after fumigation, is evidenced by a rather rapid disappearance of chlorophyll from an otherwise apparently normal leaf. The acute type sometimes results in markings of the alfalfa leaves within the hour, the leaves first stiffening and then becoming flaccid. The flaccid areas of the leaves bleach almost to an ivory color after exposure to a few hours of sunshine. The injury is first noted at the margin of the leaflet, and progresses between the veins toward the center. Leaves frequently drop from the plant when the greater part of the green tissue of the leaflets is destroyed. The injury with which this paper deals was mostly of the acute type, but there had been little, if any, dropping of the leaves at the time the alfalfa was cut.

The question has been asked by farmers and others living in areas where the alfalfa crop is sometimes affected by sulphur dioxide whether the injury to the leaflets reduces the feeding value of the alfalfa. If the feeding value is reduced it is important to know whether the reduction is due to lessened palatability, to decreased digestibility, or to an actual diminution in the nutrient value of the plant. In the fall of 1930, alfalfa fumigated by sulphur dioxide became available and the opportunity was presented to study the problem by conducting a 90-day double-reversal feeding trial.

EXPERIMENTAL MATERIALS AND METHODS

A field of fourth-cutting alfalfa which had been noticeably marked by sulphur dioxide on three occasions and subjected to relatively high concentrations on numerous other occasions, was selected for the experiment. The alfalfa was grown between the approximate dates of August 3 and September 8, 1930, on a 20-acre field in the upper Rio Grande Valley, situated about 4 miles northwest of the plant of the El Paso Smelting Works at El Paso, Tex.

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[2] The authors acknowledge the assistance of the following persons during the course of the experiment: L. V. Olson, for the photograph shown as fig. 3 and data for the curves in fig. 2; Dr. M. D. Thomas, for sulphur analyses of the two hays; G. R. Quesenberry, for grading the hays; Dr. J. N. Abersold, for energy determinations on the feeds, refuse, and excreta; W. H. Ball, for the data used in making fig. 1; and Fred Wolf, John Gaume, and Maggie McKeand, for assistance in making the many calculations involved.

[3] Reference is made by number (italic) to Literature Cited, p. 391.

Before the alfalfa to be used for the experiment was cut, the percentage of the leaflets that were marked by the gas was determined. The calculations were made from the averages of actual counts of all the marked and all the normal leaflets within the area of a quadrat of 4 square feet at definite intervals diagonally through the field, and from estimates based on these counts. The injury varied greatly in different parts of the field, as is shown in figure 1. Variation in the

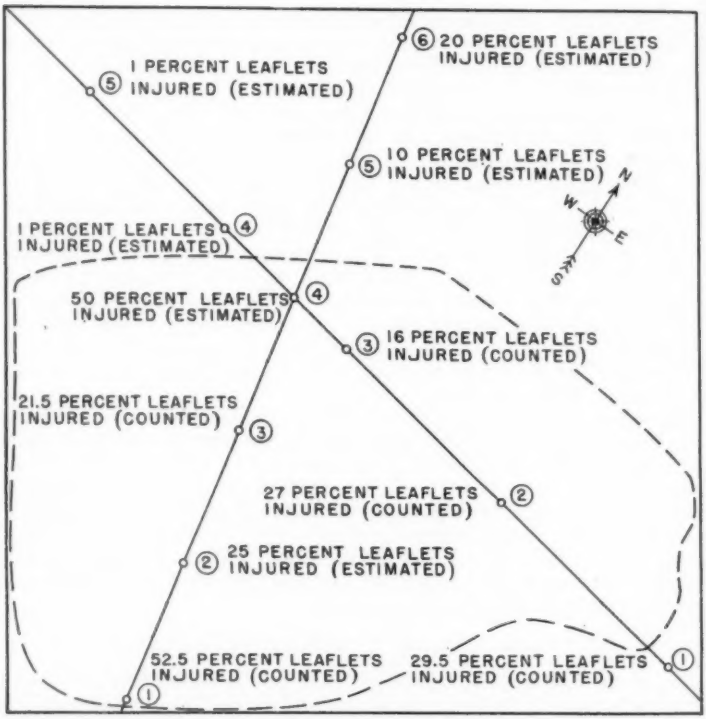
⑥ 20 PERCENT LEAFLETS INJURED (ESTIMATED)
① PERCENT LEAFLETS INJURED (ESTIMATED) ⑤
10 PERCENT LEAFLETS INJURED (ESTIMATED) ⑤
N
W
E
S
① PERCENT LEAFLETS INJURED (ESTIMATED) ④
50 PERCENT LEAFLETS INJURED (ESTIMATED) ④
16 PERCENT LEAFLETS INJURED (COUNTED) ③
21.5 PERCENT LEAFLETS INJURED (COUNTED) ③
27 PERCENT LEAFLETS INJURED (COUNTED) ②
② 25 PERCENT LEAFLETS INJURED (ESTIMATED)
52.5 PERCENT LEAFLETS INJURED (COUNTED) ①
29.5 PERCENT LEAFLETS INJURED (COUNTED) ①

FIGURE 1.—Alfalfa field, showing location of points at which injury was estimated and area (within dotted lines) from which the experimental hay was obtained.

intensity of injury is not unusual even in limited areas, and is sometimes very marked over larger areas. This is thought to be due to air currents which vary the concentration of the gas, to the density of vegetation which absorbs the gas, and to differences in soil moisture. The alfalfa used for the experiment was taken from the south portion of the field only (the area within the dotted lines in fig. 1), where more than 25 percent of the leaflets were acutely marked and the injury was most conspicuous.

The smelter was in continuous operation while the experimental alfalfa was growing, and during a large part of the time the wind was from the southeast and carried sulphur dioxide to the field. Records from an automatic sulphur dioxide recorder (7) located about 0.7

mile nearer the smelter stack, are believed to show the approximate durations of exposure and concentrations of sulphur dioxide to which this field was subjected (fig. 2).

Owing to a burned-out fuse, the records for August 21 and 22 are incomplete, but the field is known to have been noticeably marked

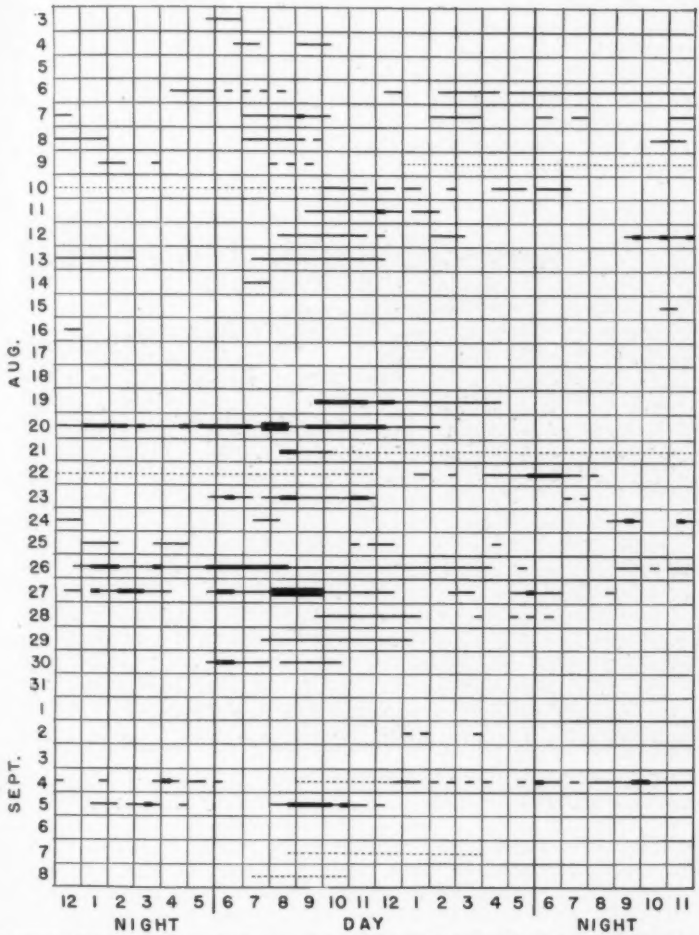

FIGURE 2.—Sulphur dioxide fumigations recorded 0.7 mile from the fumigated alfalfa field, between August 3 and September 8, 1930. Blank spaces indicate no sulphur dioxide; light lines, traces; medium lines, light concentrations; heavy lines, concentrations strong enough to mark vegetation; and dotted lines indicate recorder not working.

on those days. On August 27 the heaviest fumigation occurred. This was 12 days before the hay was cut.

Figure 3, an aerial photograph, shows the relation of the recorder and the smelter stack to the field from which the fumigated alfalfa

was obtained. It may be noted that the recorder is nearly on an air line between the south corner of the field and the smelter stack. The photograph shows also the type of topography surrounding this narrow cultivated valley, which in part accounts for the varied air currents.

For control purposes, fourth-cutting alfalfa grown during the same period in a field more than 30 miles north of the smelter was used. This hay was selected by a Government hay grader to match the fumigated hay as nearly as possible, except for sulphur dioxide markings. The field from which the hay was obtained is located more than 20 miles beyond the most remote point from the smelter where even traces of sulphur dioxide markings have been observed, so that it is reasonable to assume that the plants had not been subjected to sulphur dioxide gas.

FIGURE 3.—Aerial photograph showing relative positions of the fumigated field (within square), gas recorder (in circle), and smelter smokestack (indicated by arrow) in a narrow irrigated valley. The alfalfa fumigated with sulphur dioxide was obtained from within the area enclosed by the dotted line.

Both lots of alfalfa as finally selected were graded U. S. No. 2 Extra Leafy. The fumigated hay was found to consist of 42.03 percent of stems and 57.97 percent of leaves by weight. The check hay contained 46.93 percent of stems and 53.07 percent of leaves. The grader stated that the market value of the check hay was about 5 percent higher than that of the fumigated hay, principally on account of the difference in color.

The double-reversal feeding and digestion trial of 90 days' duration was conducted with 10 lactating cows. All of the cows were excellent producers. It will be noted from table 1 that five of them were not pregnant at the end of the experiment, and only one had carried a fetus for more than 68 days. Eckles (*2, pp. 413–415*) has shown that

the first 150 days of pregnancy do not materially affect the rate of milk production.

TABLE 1.—*Description of cows used in the fumigated alfalfa experiment*

Cow no.	Breed	Age at close of experiment	Duration of pregnancy at close of experiment	Time from freshening date to close of experiment	Cow no.	Breed	Age at close of experiment	Duration of pregnancy at close of experiment	Time from freshening date to close of experiment
		Days	*Days*	*Days*			*Days*	*Days*	*Days*
16	Holstein-Friesian	1,861	68	156	22	Jersey	1,647	(1)	368
31	do	1,183	162	206	45	Guernsey	867	(1)	129
42	do	958	40	232	24	do	1,500	(1)	167
41	Guernsey	975	(1)	139	32	Holstein-Friesian	1,168	50	198
18	Jersey	1,791	(1)	393	40	do	991	8	250

[1] Not pregnant.

The cows were divided into two groups of five each. One group was fed the fumigated hay and the other was fed the check hay, the hays being reversed at the end of each 30-day period. Otherwise the rations of the two groups were identical and consisted of a good grade of silage made from Mexican June corn, a grain mixture consisting of 3 parts of ground no. 2 mixed corn, 1 part of wheat bran, 1 part of cottonseed, and 1 part of cottonseed meal (43-percent protein). It is the practice in this vicinity to feed silage and a grain ration with the alfalfa, and this was done by dairymen who were concerned with alfalfa marked by sulphur dioxide.

The time was divided into three periods of 30 days each. Digestion trials were conducted during the last 10 days of each 30-day period. Three of these 10 days were allowed for the cows to become accustomed to the conditions of the digestion trials; the remaining 7 days were used as a collection period.

The alfalfa was fed to the cows at the rate of 1¾ pounds for each 100 pounds of live weight, the silage at the rate of 1½ pounds for each 100 pounds of live weight, and the grain at the rate of 1 pound for each 4 pounds of 4-percent fat-corrected milk. The proper amount of feed for each cow in relation to her weight and production of 4-percent fat-corrected milk was calculated at the beginning of the experiment, and the amount of feed was not altered during the 90 days of the experiment. During the first 20 days of each 30-day period the feeds were weighed to the closest 0.1 pound, and during the last 10 days of each 30-day period the feeds were weighed to the closest gram and the water consumed was weighed to the closest 0.1 pound. The weight of feeds fed per day for the entire 90 days and also the daily weight of dry matter in the orts during the digestion periods are shown in table 2.

At the beginning of each digestion trial, enough grain to last for the full 10 days was thoroughly mixed and sampled, and the 30 feedings for each cow were weighed into paper bags and labeled. The silage was removed from the silo shortly before it was fed and the proper quantity weighed for each cow. Representative samples of the alfalfa, which was fed whole, were secured during each digestion trial. The values for alfalfa shown in table 3 are the averages of these samples. The feeds, orts, and feces were analyzed by the official methods of the Association of Agricultural Chemists (*1*).

TABLE 2.—*Amount of feeds fed per day during entire 90 days, and dry matter in orts during digestion trials, of fumigated-alfalfa experiment*

Cow no.	Feeds fed per day			Dry matter in orts per day		Cow no.	Feeds fed per day			Dry matter in orts per day	
	Alfalfa	Silage	Grain	Fumigated alfalfa	Check alfalfa		Alfalfa	Silage	Grain	Fumigated alfalfa	Check alfalfa
	Grams	*Grams*	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
16	11,430	9,798	8,301	1,668	450	24	7,893	6,804	5,442	0	0
31	11,160	9,525	4,626	1,768	3,551	32	9,525	8,148	4,083	576	1,477
42	9,117	7,758	5,034	198	494	40	7,893	6,804	5,442	720	108
41	7,893	6,804	4,356	923	331						
18	8,301	7,077	2,859	201	493	Mean				715	845
22	7,077	6,123	3,402	722	910						
45	7,893	6,804	4,899	378	636	S. D					794
						Odds					2.11:1

The percentage of the dry matter in the feeds, and the analyses of the dry matter, are shown in table 3.

TABLE 3.—*Composition of dry matter in feeds fed during digestion trials to cows on fumigated-alfalfa experiment*

Kind of feed and period	Dry matter	Protein	Fat	Fiber	Nitrogen-free extract	Ash
Mixed grain:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Period 1	92.39	20.31	9.03	12.34	53.23	5.09
Period 2	91.35	20.83	8.67	14.02	51.39	5.09
Period 3	93.32	19.97	9.57	12.77	52.76	4.93
Silage	22.17	7.18	2.20	29.85	52.21	8.56
Alfalfa, fumigated	87.21	15.29	1.80	32.99	41.54	8.38
Alfalfa, check	85.39	13.45	1.79	36.89	39.88	7.99

Two samples from each of the lots of hay were analyzed for sulphur by the Burgess-Parr bomb method. The average sulphur content on a moisture-free basis for the check hay was 0.384 percent, and for the fumigated alfalfa, 0.617 percent.

The energy of the feeds was determined by the use of the Parr oxygen-bomb calorimeter apparatus. The Benedict oxycalorimeter was used to determine the energy value of the refuse and feces. The energy in the milk was calculated by the method of Gaines and Davidson (*4*).

Sand was used for bedding during the 3 preliminary days of each digestion trial, and canvas mattresses filled with straw were used during the 7 collection days. Attendants were constantly present to collect the excreta. At the end of each 24-hour collection period the excreta were thoroughly mixed and an aliquot portion taken for a composite sample.

Throughout the 90 days of the experiment the cows were weighed between 10 and 11 a. m., and while on digestion trial, at 5 a. m. also. During the digestion period the cows were exercised for 30 minutes by walking 1.25 miles immediately after the 10 a. m. weighing.

The milk was weighed at each milking during the entire 90 days and a 5-day composite sample was secured for butterfat analysis by the Babcock method.

EXPERIMENTAL RESULTS

MILK PRODUCTION

The results secured, as measured by the production of 4-percent-fat-corrected milk while the two hays were consumed, are shown in table 4. The productions during the second 10 days of the first and the third 30-day periods were averaged. This average was compared with the production during the second 10 days of the second 30-day period, in order to compensate for an advancing lactation period. The average production of 4-percent fat-corrected milk per cow while the cows were fed fumigated hay was 345.6 pounds, and while they received the check hay, it was 341.1 pounds. When the data are analyzed by Student's method (*6*), the odds are 2.68:1 that the difference is significant. It is apparent that there was no significant difference in the results secured with the two rations as fed in this experiment.

Data for the milk produced during the rest of the experimental period are not presented in detail since they were concordant with the data presented in table 4. The total production of fat-corrected milk during the portions of the 90-day trial that the 10 cows received fumigated hay was 15,508.3 pounds and during the portions that they received the check hay, 15,151.5 pounds. When the production of the first and the production of the third 30-day period were averaged and compared with the production of the second 30-day period, it was found that 10,276.0 pounds of 4-percent fat-corrected milk was produced while the fumigated hay was fed, and 10,129.2 pounds while the check hay was fed, a difference 0.1 percent greater than that shown by the data in table 4.

TABLE 4.—*Milk production while cows received alfalfa hay fumigated with sulphur dioxide compared with production while cows received nonfumigated hay during 10-day periods of a double-reversal feeding trial*

Cow no.	4-percent fat-corrected milk during fumigated hay periods	4-percent fat-corrected milk during check hay periods	*D*	D^2	Cow no.	4-percent fat-corrected milk during fumigated hay periods	4-percent fat-corrected milk during check hay periods	*D*	D^2
	Pounds	*Pounds*				*Pounds*	*Pounds*		
16	603.93	553.76	50.17	2,517.03	45	380.18	369.28	10.90	118.81
31	286.25	310.32	−24.07	579.36	24	377.00	396.38	−19.38	375.58
42	371.88	367.75	4.13	17.06	32	315.02	307.13	7.89	62.25
41	305.08	326.40	−21.32	454.54	40	426.90	407.72	19.18	367.87
18	160.50	167.03	−6.53	42.64					
22	229.55	204.72	24.83	616.53	Total	3,456.29	3,410.49	45.80	5,151.67
					Average	345.63	341.05	4.58	515.167

By Student's method: $S.D. = \sqrt{515.167 - (4.58)^2} = 22.23$

$$\frac{M}{S.D.} = Z = \frac{4.58}{22.23} = 0.21$$

Odds=2.68:1 that there is a significant difference. Odds of 25 or 30 to 1 are required to establish a definite difference.

WEIGHTS

A 3-day running average of the weights of each cow revealed considerable variation in fill and shrink incident to stabling for digestion trials, but there were no significant actual gains or losses in weight

during the 90 days. During the 7-day collection periods, each of the cows was weighed at 5 a. m., the weights at both the beginning and the end of each period being included, which gives a total of eight for each cow for each digestion trial. The average weight of the cows while receiving the fumigated hay was 1,079.8 pounds, and while receiving the check hay, 1,078.2 pounds—a difference of only 1.6 pounds.

PALATABILITY

The rations contained a relatively large quantity of alfalfa and the cows received as much alfalfa as they would consume, or slightly more so that the orts from the two hays might indicate their comparative palatability. In most cases the material refused was alfalfa stems. During the second digestion trial, cow 31 was off feed for a few days and passed a small amount of blood with the urine. This apparently accounts for her high feed refusal during this period.

The dry matter in the orts was determined for the periods that the cows were on digestion trials. The results are shown in table 2. The mean weight of dry matter in the orts while the cows received fumigated hay was 715 g. per day and while they received the check hay, 845 g.

The dry matter in the orts during the first 20 days of each 30-day period was not determined, but the daily weights of orts were practically the same as during the digestion periods.

These results, as well as observations made when rations were changed, indicate no difference in palatability of the two rations.

APPARENT DIGESTIBILITY OF THE COMPLETE RATIONS

The apparent digestibility of the dry matter, energy, protein, ether extract, fiber, nitrogen-free extract, and ash is shown in table 5. In no case was there a significant difference between the two rations.

The correlation (*8*) between the apparent digestibility of the energy and of the dry matter in the 30 trials (0.9441) was very high. The high correlation is clearly indicated in figure 4.

TABLE 5.—*Apparent digestibility of the various constituents of entire rations containing fumigated and nonfumigated alfalfa*

Cow no.	Dry matter		Energy		Protein		Ether extract		Fiber		Nitrogen-free extract		Ash	
	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
16	64.6	66.6	65.0	67.8	70.6	70.8	89.6	93.0	46.7	52.5	71.7	74.2	46.4	44.1
31	65.7	71.1	65.2	71.3	69.1	75.5	87.2	88.7	52.9	63.6	72.7	75.4	45.6	50.4
42	65.2	65.1	64.0	66.3	70.1	68.9	87.0	87.8	51.2	54.7	72.3	72.3	45.2	38.1
41	65.6	62.8	65.8	63.7	69.6	70.6	86.7	87.7	53.0	51.2	72.5	70.5	42.1	29.4
18	65.1	64.0	64.5	63.8	71.6	70.2	84.6	78.3	54.2	54.4	71.4	71.0	43.8	41.4
22	57.6	60.5	58.8	61.1	67.2	63.1	71.9	84.1	40.9	48.9	67.3	68.7	26.4	32.2
45	65.7	63.3	66.4	63.8	72.5	67.0	88.2	83.1	53.9	49.9	73.4	71.3	29.6	38.2
24	69.3	65.8	69.2	65.4	74.7	69.4	83.6	83.5	55.5	50.5	77.6	74.3	44.3	48.3
32	64.4	67.3	64.2	66.9	72.1	70.7	91.5	80.2	53.1	61.8	70.4	72.4	38.5	44.5
40	67.1	67.0	67.9	67.8	75.8	71.5	90.3	85.9	50.4	57.9	74.8	72.9	39.7	39.4
Mean	65.0	65.3	65.1	65.8	71.3	69.8	86.1	85.2	51.2	54.5	72.4	72.3	40.2	40.6
S. D		3.21		2.87	3.41		6.07			5.25	2.03			6.37
Odds		1.6:1		2.9:1	8.3:1		2.2:1			18.8:1	1.0:1			1.1:1

PERCENT OF ENERGY INTAKE RETURNED IN MILK

The energy in the milk produced by the cows while on the digestion trials was determined by converting the milk production to the equivalent amount of 4-percent fat-corrected milk (*4*). The percentages of energy intake returned in the form of milk when the two rations were fed were determined and compared by Student's method as shown in table 6. The means are 18.65 percent for the fumigated-alfalfa periods and 18.49 percent for the check-alfalfa periods. This difference is not

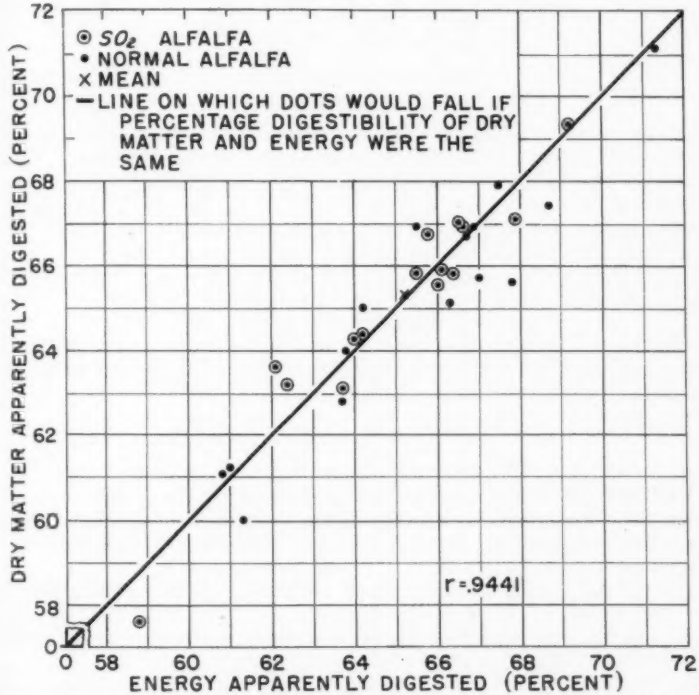

FIGURE 4.—Correlation between the apparent digestibility of the dry matter and of the energy in the total ration. The ration consisted of alfalfa, corn silage, and a grain mixture, and was fed to 10 cows during each of 3 digestion trials.

significant. Forbes and Voris (*3*) show an average return of 20.76 percent of the energy fed to 12 cows throughout the duration of their lactation periods. The variations in their experiments were between 18.04 and 23.35 percent for the individual cows. It may be pointed out that the rations fed to the cows in the experiment of Forbes and Voris were reduced as the lactation advanced to correspond to the production of the individual cows.

TABLE 6.—*Energy intake returned in milk from cows fed rations cotaining fumigated and nonfumigated alfalfa*

Cow no.	Fumigated alfalfa	Check alfalfa	Cow no.	Fumigated alfalfa	Check alfalfa
	Percent	*Percent*		*Percent*	*Percent*
16	22.42	21.87	32	18.78	18.24
31	15.46	18.12	40	23.42	22.58
42	19.37	20.07			
41	17.76	16.33	Mean	18.65	18.49
18	11.59	10.37			
22	15.52	16.57	S. D.	1.2158	
45	22.05	20.83	Odds	1.83:1	
24	20.17	19.95			

pH VALUE OF THE URINE

Every morning while the digestion trials were in progress, a sample of approximately 100 cc of urine was secured from each cow. When the temperature of the samples had reached equilibrium with the temperature of the room (between 60° and 70° F.) in which the potentiometer was located, the pH value was determined. A Leeds and Northrup 7655 potentiometer and a quinhydrone electrode were used. The pH values ranged from 8.22 to 8.43 while the cows were fed the fumigated alfalfa, and from 8.18 to 8.57 while they were fed the check alfalfa. The respective approximate means were 8.30 and 8.38. While the pH value of the urine tended to be lower when the ration contained the alfalfa with the higher quantity of sulphur, the odds of 8.33:1 indicate no significant difference.

SUMMARY

In a 90-day double-reversal feeding trial, 10 purebred cows, Holstein-Friesians, Jerseys, and Guernseys, were fed a ration of mixed grain, corn silage, and alfalfa that had been injured by sulphur dioxide gas. For comparison the same ration was fed, except that nonfumigated alfalfa was used.

The average daily production of 4-percent fat-corrected milk per cow was found to be 34.6 pounds while the cows received the fumigated alfalfa, and 34.1 pounds while they received the check alfalfa during the second 10 days of each 30-day period.

There were no significant changes in the weights of the cows.

The last 10 days of each 30-day period were used to determine the apparent digestibility of the two rations. No significant differences were found in the apparent digestibility of the various constituents of the rations when the results from feeding the two hays were compared. A correlation of 0.9441 was found between the apparent digestibility of the dry matter and the apparent digestibility of the energy.

During the digestion trials the average daily refusal of dry matter was 715 and 845 g per cow while receiving the fumigated alfalfa and the check alfalfa, respectively. The difference is not significant.

Of the ingested energy, 18.65 percent was returned in milk while the cows were receiving the fumigated alfalfa, and 18.49 percent while they were receiving the check alfalfa. The difference is not significant.

While the pH value of the urine voided by the cows when receiving the fumigated alfalfa was slightly lower than when receiving the check alfalfa with its somewhat lower sulphur content, the difference is not significant.

CONCLUSION

No significant increase or decrease in the feeding value of alfalfa hay made from alfalfa subjected to a sufficient amount of sulphur dioxide to cause acute injury to more than 25 percent of the leaflets was apparent under the conditions of this experiment.

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CERTAIN RELATIONSHIPS BETWEEN THE CALCIUM AND OXALATE CONTENT OF FOLIAGE OF CERTAIN FOREST TREES [1]

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INTRODUCTION

That calcium oxalate crystals exist in the tissues of many plants is a well-established fact. Little investigation, however, has been conducted to determine what proportion of the calcium is present as the oxalate, or what percentage of the oxalate ions are combined with calcium. This paper shows in what form the oxalate ions exist in the leaves of certain forest trees and indicates certain associations between the calcium content and the oxalate content of the leaves.

No attempt is made at this time to formulate a theory which associates the calcium requirements of forest trees with the production of oxalic acid. Such a relationship may exist, but the data included in this paper do not adequately substantiate such a theory. Further studies are being conducted on this problem.

EXPERIMENTAL METHODS

The trees from which the leaf samples were picked were growing in the vicinity of Ithaca, N. Y., on Dunkirk silty clay loam and Lordstown stony silt loam. The Dunkirk soil is derived from glacial lake-laid deposits, and has an acid surface soil, but a calcareous subsoil. The Lordstown soil is derived from glacial till composed of fragments of shale and sandstone. It is acid throughout the profile.

The leaf samples were secured between September 10 and 15, 1936, according to the technique described by Mitchell.[2] The samples were dried for 24 hours at a temperature of 70° C., and subsequently ground so that all of the material passed through a 40-mesh sieve.

In the determination of total calcium, a ½-g sample was ignited in an electric muffle furnace at a temperature of approximately 800° C. The ash was taken up with hydrochloric acid and transferred quantitatively to a 150 ml beaker. The calcium was precipitated as the oxalate by the usual method, and titrated with 0.05 normal potassium permanganate solution.

The total oxalate content of the tissues was determined by the ether extraction method of Pucher, Vickery, and Wakeman.[3]

The acetic acid-insoluble calcium was determined as follows: A 25-ml portion of normal acetic acid solution was added to a 1-g leaf

[1] Received for publication June 17, 1937; issued September 1937.

[2] MITCHELL, H. L. TRENDS IN THE NITROGEN, PHOSPHORUS, AND POTASSIUM CONTENT OF THE LEAVES OF SOME FOREST TREES DURING THE GROWING SEASON. Black Rock Forest Papers 1, no. 6, illus. 1936.

[3] PUCHER, G. W., VICKERY, H. B., and WAKEMAN, A. J. DETERMINATION OF THE ACIDS OF PLANT TISSUE. II. TOTAL ORGANIC ACIDS OF TOBACCO LEAF. Indus. and Engin. Chem., Analyt. Ed. 6: 140-143. 1934.

sample and the mixture was agitated on a mechanical shaker for 10 minutes and then filtered on asbestos by the use of suction. It was next washed with successive 25-ml portions of the acetic acid solution until somewhat more than 200 ml had passed through the filter. The filtrate was then transferred to a 250 ml volumetric flask. A 50-ml aliquot was evaporated to dryness and the calcium content determined in the same manner as described above. This value, the acetic acid-soluble calcium, was subtracted from the total calcium content to determine the amount which was insoluble in acetic acid. It was assumed that this fraction corresponded to calcium in the form of calcium oxalate.

EXPERIMENTAL RESULTS

The total calcium content, total oxalate content, and acetic acid-insoluble calcium of the foliage of various tree species are presented in table 1. The results are expressed as milliequivalents per 10 g of oven-dried material. The percentage of calcium insoluble in acetic acid and the series designation of the soils upon which the trees were growing are also reported. The tree species are listed in the order of their increasing total calcium content.

TABLE 1.—*Total calcium content, total oxalate content, and acetic acid-insoluble calcium content of foliage of various forest trees*

Tree species	Soil series	Total calcium per 10 g	Total oxalates per 10 g	Calcium insoluble in acetic acid per 10 g	Portion of total calcium insoluble in acetic acid
		Milli-equivalents	*Milli-equivalents*	*Milli-equivalents*	*Percent*
White pine, *Pinus strobus* L. (new needles)	Dunkirk	2.65	1.55	1.53	57.7
Red oak, *Quercus borealis* var. *maxima* (Marsh.) Ashe	Lordstown	5.20	2.47	2.45	47.2
Chestnut oak, *Quercus montana* Willd	do	6.00	3.21	3.25	54.2
Yellow birch, *Betula lutea* Michx	do	7.51	4.45	4.50	59.9
Sugar maple, *Acer saccharum* Marsh	do	7.95	4.75	4.70	59.1
White cedar, *Thuja occidentalis* L. (new leaves)	Dunkirk	8.75	6.01	5.99	68.4
Red cedar, *Juniperus virginiana* L. (new leaves)	do	8.85	6.55	6.60	74.6
White ash, *Fraxinus americana* L	Lordstown	10.05	5.51	5.65	56.2
White cedar (old leaves)	Dunkirk	11.30	8.54	8.55	75.6
Ironwood, *Ostrya virginiana* (Mill.) Koch	Lordstown	13.10	8.51	8.60	65.6
Trembling aspen, *Populus tremuloides* Michx	do	13.24	5.71	5.52	41.6
Do	Dunkirk	13.30	6.41	6.43	48.3
Pignut hickory, *Hicoria glabra* (Mill.) Sweet	Lordstown	13.31	12.69	11.52	86.6
Red cedar (old leaves)	Dunkirk	14.81	13.62	11.81	79.7
Pignut hickory	Lordstown	16.85	16.12	14.98	88.9
Basswood, *Tilia americana* L	do	18.13	10.91	10.92	60.2

There was a distinct tendency for the total oxalates to be correlated with the total calcium content. Trembling aspen and basswood were outstanding exceptions to this rule, and white ash and ironwood were somewhat out of line. The total oxalates did not exceed the total calcium in any case.

A comparison of the total oxalates and acetic acid-insoluble calcium shows a close agreement in nearly all cases. This seems to support the conclusion that in most cases all of the oxalate ions were combined with calcium. Pignut hickory and red cedar were the only two exceptions to this rule.

An examination of the last column of table 1 shows that the percentage of the total calcium present as calcium oxalate varied con-

siderably. The two aspens and red oak were lowest in this respect. The white cedar, red cedar, and pignut hickory were highest. There was a close agreement between the figures for the two aspen samples, as well as for the two hickory samples, although each tree was growing on a different site. The data also indicate that when the calcium oxalate content reaches a certain value (79 percent or above) in relation to the total calcium any oxalic acid formed in excess of that amount will be in some form other than calcium oxalate.

DISCUSSION

The fact that the calcium content always exceeded an equivalent amount of oxalates is of particular interest. Of course, if all the oxalates were present as calcium oxalate, the above situation would have to exist. The question arises, however, as to why the production of oxalic acid in the leaves was maintained within such limits as to prevent it from exceeding the calcium content, or, conversely, why the absorption of calcium was limited to such an extent that it did not exceed the oxalate content to any great degree. Parker and Truog [4] advanced the theory that the amounts of calcium and nitrogen in plant tissues are highly correlated because of the fact that protein metabolism is one of the chief sources of organic acids and the calcium absorbed would function in the neutralization of these acids.

The total nitrogen content of the leaves used in this study was determined. There was essentially no relationship between the nitrogen and the oxalate content. For example, the old red cedar leaves contained only 1.34 percent of nitrogen, yet the oxalate content was 13.62 milliequivalents. Red oak, on the other hand, had a nitrogen content of 2.05 percent, with an oxalate content of only 2.47 milliequivalents. It would appear, therefore, that the production of oxalic acid in the forest tree leaves used in this study was dependent upon more factors than simply the nitrogen content. Furthermore, Dunne [5] has indicated that calcium does not necessarily function in the neutralization of organic acids, since other bases such as potassium are sufficient for the process.

Another factor which complicates the explanation of these results is that two species, namely, cucumbertree, *Magnolia acuminata* L., and tuliptree, *Liriodendron tulipifera* L., did not contain any oxalates. They had a calcium content of 7.70 and 15.15 milliequivalents, respectively. All of the calcium in each case was soluble in normal acetic acid solution. It is of interest to note that cucumbertree and tuliptree belong to the same botanical family, which further indicates that the production of oxalic acid by plants may be an inherent characteristic, and is not necessarily influenced by environment.

If the absorption of calcium is strongly influenced by the precipitation of calcium as calcium oxalate within the plant, one would expect those species which produce no oxalic acid to contain relatively small amounts of calcium. But, as has been shown above, such may not be the case. There are other forms of water insoluble calcium, of course, which would be soluble in acetic acid and would not be revealed in this study. Such forms might be more abundant in those

[4] PARKER, F. W., and TRUOG, E. THE RELATION BETWEEN THE CALCIUM AND THE NITROGEN CONTENT OF PLANTS AND THE FUNCTION OF CALCIUM. Soil Sci. 10:49–56, illus. 1920.

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species which had a high calcium content with small amounts of oxalates or none. Additional study is needed on this point.

The fact that, with the exception of two species, essentially all of the oxalates could be accounted for as calcium oxalate is interesting. This is not true of certain other plants. Lebedeva and Pochinok,[6] working with sugar-beet leaves, showed that water-soluble oxalates were present in quantities up to five times the amount of calcium oxalate, and, moreover, they found no water-soluble or acetic acid-soluble calcium. Dunne has shown that buckwheat plants may contain in equivalent amounts nearly twice as much oxalate as calcium. His data indicated that oxalic acid could be precipitated in a relatively water-insoluble form by either calcium or potassium, the latter case resulting in the formation of potassium acid oxalate.

The fact that the calcium content of the older leaf tissues, in the case of the cedars, was greater than the oxalate content, even though the oxalate content had increased over that of the younger tissues, again showed a distinct relationship between calcium and oxalates. Whether the calcium content was related to the oxalate content or whether outside factors influenced both values, is a matter for conjecture. It does not seem likely however, that these relationships were entirely accidental.

Further work is needed on the forms of water-insoluble calcium, on the metabolic availability of calcium as calcium oxalate, and upon the actual calcium requirements of forest tree species.

SUMMARY

A study has been made of the relationship between the calcium and the oxalate content of the foliage of various forest trees.

The total oxalates in the leaves were, in general, found to be correlated with the total calcium content.

In none of the species studied did the total oxalates exceed the total calcium content.

Except in two species, all of the oxalates were present as calcium oxalate.

The leaves of certain forest trees may not contain any oxalates, yet the calcium content may be high.

[6] LEBEDEVA, A. O., and POCHINOK, K. N. THE DETERMINATION OF THE FORMS OF CALCIUM AND OXALIC ACID IN LEAVES OF SUGAR BEETS. Nauch. Zap. Sakharnoi Prom. 11, Book 46-8, no. 8-10. 31-47. 1934. [Abstract in Chem. Abs. 29:5884, 1935.]

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